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ON THE SPECTRUM OF THE P_2 MOLECULE[1]

BY G. HERZBERG[2], L. HERZBERG[3], AND G. G. MILNE[4]

Abstract

Five bands of the ultra-violet system of P_2, with low v' and v'' values, have been measured under large dispersion and analysed. They serve to determine much more accurately than heretofore possible the rotational constants B_e'' and α_e'', the moment of inertia I_e'', and the internuclear distance r_e'' of the P_2 molecule in the ground state. The following values have been found: $B_e'' = 0.3031$ cm.$^{-1}$, $\alpha_e'' = 0.00138$ cm.$^{-1}$, $I_e'' = 92.36 \cdot 10^{-40}$ gm.-cm.^{2}, $r_e'' = 1.895 \cdot 10^{-8}$ cm.

Introduction

The spectrum of the diatomic phosphorus molecule is known to consist of two band systems, one very extensive and prominent system extending from 3200 to 1800 Å and another weak system in the visible region. While the latter has not yet been investigated in any detail, the former has been the subject of a number of investigations (1, 2, 4). It represents a $^1\Sigma_u^+ - {}^1\Sigma_g^+$ transition, the lower $^1\Sigma_g^+$ level being the ground state of the P_2 molecule. However, owing to the large moment of inertia of the molecule and the considerable overlapping of various bands, up to now only the rotational structure of bands with large v' and v'' values has been analysed. The values for the rotational constants, moments of inertia, and internuclear distances in the vibrationless states therefore could be obtained only by means of rather long extrapolations. For example, the v'' values of the bands analysed up to the present time range from $v'' = 22$ to $v'' = 32$. There is therefore a considerable uncertainty in the B_e, I_e, r_e values. The writers thought it to be of importance to remove this uncertainty by analysing some bands with *lower* v' and v'' values.

Experimental

Spectrograms of the P_2 system in absorption were taken in the second order of our new six metre grating spectrograph. The absorption tube containing the phosphorus vapour was 2 m. long and was kept at a temperature of about 950° C., while the vapour pressure was regulated by means of a side-tube which was at a temperature between 50° and 100° C. In this way most of

[1] *Manuscript received May 13, 1940.*
Contribution from the Department of Physics, University of Saskatchewan, Saskatoon, Sask.

[2] *Research Professor of Physics.*

[3] *Ph.D.*

[4] *Graduate Student.*

the P_4 was dissociated into P_2 [see a table in Mellor (6, p. 757)]. The light passed twice through the absorption tube. A hydrogen discharge tube was used as a source of the continuous spectrum. In this way the bands 0–5, 0–6, 0–7 were obtained in absorption with sufficient intensity to make an analysis possible.

In addition, emission spectrograms of the bands 2–15 and 3–16 taken many years ago with a 3 m. grating were analysed.

Rotational Analysis

In Table I are given the wave numbers of the lines in the five bands that have been measured. Since the region near the heads and origins of the bands is not resolved, the numbering of the lines in these bands is not obvious. In the case of the three bands 0–5, 0–6, 0–7 measured in absorption, which have the upper state in common, the numbering was found by systematic trial [see for example Herzberg (3)] until the combination differences for the upper state, $\Delta_2F'(J)$, agreed exactly. Although none of the bands 2–15, 3–16 measured in emission has a state in common with any other band whose fine structure has been analysed, the numbering could be found fairly easily since the approximate $\Delta_2F(J)$ values were known from the other bands. In each case only one numbering fulfilled all necessary conditions.

TABLE I

WAVE NUMBERS OF BAND LINES

J	0 – 5 ν_0 = 42969.92		0 – 6 ν_0 = 42223.39		0 – 7 ν_0 = 41483.56		2 – 15 ν_0 = 36713.85*		3 – 16 ν_0 = 36484.56	
	R	*P*	*R*	*P*	*R*	*P*	*R*	*P*	*R*	*P*
5									36485.96	36480.79
6										
7									485.96	478.89
8		42961.93								
9		960.45								476.46
10		958.86								
11		957.11			41482.42	41471.45			484.51	473.74
12		955.30								
13		953.36			480.94	467.97			483.33	470.75
14		951.51	42219.11	42205.75						
15		949.59	218.17	203.50	478.89	463.95			481.85	467.23
16		947.46	217.05	201.36						
17	42961.93	945.13	215.93	199.04	476.21	459.69			480.06	463.54
18	960.45	942.65	214.39	196.78						
19	958.86	940.17	212.90	194.16	473.69	454.73			477.74	459.39
20	957.11	937.27	211.15	191.64		451.41				
21	955.30	934.50	209.46	188.95	470.13	449.63			475.15	454.90
22	953.36	931.82	207.62	186.17		446.26				
23	951.19	928.78	205.75	183.16	466.39	443.86			472.25	450.17
24	949.23	925.63	203.50	180.22		440.33				
25	946.77	922.45	201.36	177.12	462.38	437.82			469.02	444.94
26	944.77	918.90	199.04	173.79		434.51				

* *Extrapolated from lines with high J values.*

TABLE I—*Concluded*

WAVE NUMBERS OF BAND LINES—Concluded

J	0 – 5		0 – 6		0 – 7		2 – 15		3 – 16	
	R	P	R	P	R	P	R	P	R	P
27	942.13	915.72	196.78	170.34	458.07	431.55			465.46	439.60
28	939.66	912.03	194.16	167.21		428.00				
29	936.96	908.68	191.64	163.46	453.05	424.70	36692.67(?)		461.40	433.86
30	934.12	904.36	188.95	159.83	450.56	421.16				
31	931.42	900.66	186.17	156.11	447.84	417.37	687.89(?)		457.24	427.64
32	928.36	896.88	183.16	152.11	445.01					
33	925.04	892.94	180.22	148.14	442.05	409.78	682.80(?)		452.70	421.10
34	922.03	888.68	177.12	144.41	439.17					
35	918.55	884.33	173.79	139.97	435.93	401.80	677.44	36644.35	447.71	414.27
36	915.08	879.49	170.34	135.45	432.80	397.23				
37	911.47	875.56	167.21	130.94	429.47	393.22	672.19	636.97	442.36	407.13
38	42908.07	42870.83	42163.46	42127.20	41425.83	41388.55				
39	903.61	866.21	159.83	121.99	422.15	384.37	36666.43	36629.21	36436.75	36399.64
40	900.07	861.70	156.11	117.19	418.98	380.10				
41	896.18	856.44	152.11	112.39	414.67	375.10	660.40	621.02	430.74	391.75
42	892.22	851.30	148.14	107.29	411.23					
43	887.71	845.97	144.41	102.20	406.96		653.69	612.79	424.43	383.40
44	883.05	840.89	139.97	097.03						
45	878.26	834.98	135.45	091.80	398.68	355.20	647.11	604.13	417.69	374.89
46	874.26	829.64	130.94	086.40	394.53					
47	869.58	824.11	127.20	081.02	390.07		639.97	595.17	410.71	365.97
48	864.82	818.65	121.99	075.46	385.69					
49	859.95	812.57	117.19	069.54	381.02	333.48	632.71	585.75	403.33	356.70
50	855.05	806.15	112.39	063.77	376.26					
51	849.86	800.26	107.29	057.95	371.26	322.09	624.82	576.22	395.54	347.17
52	844.55	794.13	102.20	052.04	366.25					
53	838.94	787.94	097.03	045.72	361.27	310.02	616.70	566.22	387.45	337.26
54	833.07	781.46	091.80	039.35						
55	827.84	774.47	086.40	033.05	350.60	297.73	608.16	556.01	379.13	327.09
56	821.99	767.89	081.02	026.49						
57	816.00	761.02	074.62	020.02	339.47	284.95	599.56	545.22	370.31	316.42
58	810.31	754.31	068.76	013.34						
59	803.80	747.31	062.93	006.48	328.21	271.68	590.45	534.26	361.22	
60	797.37	740.08	056.70			264.56				
61	791.10	732.76	050.74	41992.28	316.27	258.06	580.93	522.96	351.68	
62	784.77	725.17	044.24	984.99		251.00				
63	778.07	717.89	037.92	977.72	303.86	243.75	571.08	511.22	341.87	
64	771.16	709.95	031.02	970.14		236.50				
65	764.35	702.24	024.48	962.59	290.99	228.88	561.05	499.37	331.66	
66		694.62	017.44	954.72		221.79				
67	749.64	686.13	010.28	946.52	277.54	213.47	550.48		321.06	
68	742.65	678.16				205.52				
69	734.64	669.30	41995.44	930.50	262.91	197.75	539.66		310.19	
70		(657.87)			256.49	189.24				
71		651.67	979.84	913.08	247.86	181.04	528.41			
72	42709.95	643.08			238.36	172.02				
73	701.03	633.39	962.59	895.17	230.78	163.16	516.92			
74						154.66				
75					213.47	144.64	505.05			
76						137.57				
77						124.32	492.83			

Since the fine structure is very narrow, the overlappings of lines are rather numerous. Furthermore, there is an intensity alternation 1 : 3 [see (1) and (5)], the lines with odd J values being the stronger ones. In the weaker

parts of the absorption bands and in the whole of the emission bands, only the strong lines could be measured.

In the 2–15 band, while the lines with J' values >46 follow the usual run those with J' values <46 show an increasing deviation, and they could not be observed very much further down. The reason is obviously a strong perturbation of the lines with lower J' values. Indeed in the vibrational analysis from measurements of heads, Herzberg (2) found that all bands with $v' = 2$ show a deviation of about 7 cm. $^{-1}$ from the smooth formula. An extrapolation of the head of the 2–15 band from the unperturbed lines with large J values gives a position that differs by about 7 cm.$^{-1}$ from the observed position of the head and in the expected direction. Thus, what was considered previously (2) as a vibrational perturbation actually is a strong perturbation of the first 45 rotational levels.

TABLE II

ROTATIONAL CONSTANTS OF THE LOWER AND UPPER STATES OF THE ULTRA-VIOLET P_2 BANDS

v''	B_v'' (cm.$^{-1}$)			D_v'' (cm.$^{-1}$)
	New	Ashley (1)	Herzberg (2)	
5	0.2953_8			$2.0 \cdot 10^{-7}$
6	0.2938_6			
7	0.2922_9			
15	0.2800			$1.8 \cdot 10^{-7}$
16	0.2787			
22		0.2686_9		
23		0.2671_5		
27		0.2602_5	0.2606_9	
28		0.2590_1	0.2587_1	$1.7 \cdot 10^{-7}$
29			0.2566_8	
30			0.2546_3	
31			0.2529_5	
32			0.2515_7	

v'	B_v' (cm.$^{-1}$)			D_v' (cm.$^{-1}$)
	New	Ashley (1)	Herzberg (2)	
0	0.2408			$2.5 \cdot 10^{-7}$
2	0.2374			
3	0.2360			
6		0.2309_1		
8		0.2273_0	0.2274_8	
9		0.2260_6	0.2255_3	$2.4 \cdot 10^{-7}$
10			0.2239_0	
11			0.2222_0	

From the combination differences Δ_2F' and Δ_2F'' (not given here) the rotational constants B_v' and B_v'' of the P_2 bands were calculated in the usual way, assuming calculated D values obtained from preliminary B's and ω's [see e.g., Herzberg (3)]. They are given together with the B_v values of Herzberg (2)

and Ashley (1) in Table II. They can be represented by the following two formulae

$$B_v'' = 0.3031 - 0.00138\,(v'' + \tfrac{1}{2}) - 0.0000064\,(v'' + \tfrac{1}{2})^2$$
$$B_v' = 0.2415 - 0.00153\,(v' + \tfrac{1}{2}) - 0.000014\,(v' + \tfrac{1}{2})^2$$

that is, $B_e'' = 0.3031$, $(B_0'' = 0.3024)$, $\alpha_e'' = 0.00138$, $B_e' = 0.2415$, $(B_0' = 0.2407)$, $\alpha_e' = 0.00153$ cm.$^{-1}$ Since these rotational constants are now based on bands of low v values as well as on those with high v values, they should be more reliable than those heretofore available The resultant values for the moments of inertia and the internuclear distances are

$I_e'' = 92.36 \cdot 10^{-40}$ gm.-cm.^{2}	$I_e' = 115.92 \cdot 10^{-40}$ gm.-cm.^{2}
$r_e'' = 1.895 \cdot 10^{-8}$ cm.	$r_e' = 2.123 \cdot 10^{-8}$ cm.
$(r_0'' = 1.897 \cdot 10^{-8}$ cm.)	$(r_0' = 2.127 \cdot 10^{-8}$ cm.)

These values are based on recent values for the atomic constants e, h, and N.

Acknowledgments

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A FLUID SYSTEM FOR TRANSFERRING HEAT OVER SMALL TEMPERATURE GRADIENTS WITHOUT FORCED CIRCULATION[1]

By W. H. Cook[2] and T. A. Steeves[3]

Abstract

An enclosed system of piping partly filled with liquid ammonia was found to transfer useful quantities of heat to a bunker containing a solid refrigerant (ice), with temperature gradients of 30 to 50° F. without the use of forced circulation. The system could be adapted to reduce spatial temperature variations and provide thermostatic control where solid refrigerants are used, as in railway refrigerator cars. Such arrangements are discussed briefly.

Introduction

In spite of the recent advances in mechanical refrigeration, such solid materials as ice, ice-salt mixtures, and dry ice are still used for preserving foodstuffs where suitable power sources are lacking or where economic considerations intervene. Such applications include the cooling of products in rural areas and railway car refrigeration. Under these conditions, temperature control is usually difficult since the mechanical refrigerator and its accessories, namely, distributed coils, circulating fans, and thermostats are lacking. It appears that the advantages of distributed cooling coils under thermostatic control could be applied to ice cooled systems under certain conditions, where a method capable of transferring sufficient heat from the space to the ice bunker could be devised. The present investigation was undertaken to determine the capacity of an enclosed system, partly filled with a volatile liquid (ammonia), to transfer heat.

The conditions in the end-bunker type of railway car may be described as indicating the problems involved. The bunker is filled with broken ice through which the air circulates and the resulting liquid drains from the bottom. In order to obtain car temperatures of 32 to 35° F. during the summer months it is necessary to add up to 15% salt to the ice, and under these conditions the temperature of the cooling mixture may reach 0° F. or lower. The air temperatures prevailing in the loaded car may then attain the following extremes: air temperature near bottom of bunker, 28 to 32° F.; above the load near the centre of the car, 45 to 50° F. Comparable figures are reported by several investigators (1, 2, 3). These figures indicate that a temperature difference of 50° F. may prevail between the warmest region in the car and the temperature of the cooling medium.

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[2] *Biochemist, Food Storage and Transport Investigations.*

[3] *Refrigerating Engineer, Food Storage and Transport Investigations.*

Recent developments have been of material assistance in reducing these spatial variations in temperature within the loading space. Cars with overhead bunkers are a big improvement (2, 6, 7) but the end-bunker type will necessarily be in use until replaced by the newer equipment. Forced air circulation by means of fans has been shown to improve conditions (1, 4) but is not yet in general use. Indirect cooling systems using brines or volatile liquids, such as that described in this study, have been used experimentally for controlling the temperature in railway cars cooled with dry ice (3, 5). Efficient operation of such systems might reasonably be expected over the relatively large temperature difference prevailing when dry ice is used as a refrigerant. When ordinary ice is used the temperature gradient would seldom exceed 50° F. and it might be reduced to 30° F. Under these conditions it seems reasonably certain that gravity circulation of a brine, which changes in density by only 0.3% over a 20° F. difference in temperature, could not accomplish the desired heat transfer at the levels prevailing in a railway car.

If an enclosed piping system were partly filled with a volatile liquid such as ammonia, heat could be transferred by evaporation in a "hot" coil and condensation in a "cold" coil. By analogy with mechanical systems the "hot" and "cold" coils may be termed the "evaporator" and "condenser" respectively. Since the latent heat of liquid ammonia is 545 B.t.u. per lb. at 30° F., this quantity of heat would be transferred for each pound of liquid evaporated. Since the vapour pressure of ammonia increases about 1 lb. per sq. in. for each °F. rise in temperature, between 20 and 30° F., it is obvious that a potentially adequate circulating pressure will prevail with a small difference between the temperature of the liquid in the evaporator and that of the liquid in the condenser. Similar conditions apply to other volatile fluids, but the favourable properties of liquid ammonia combined with its low cost appeared to justify tests with this material.

Description of Apparatus and Method

Valid estimates of the capacity of such systems, involving heat transfer surfaces exposed to convection cooling and connecting piping to resist fluid circulation, can be obtained only from tests conducted with equipment approximating the scale that would be used in practice. For this reason the test equipment was set up on a scale similar to that required in a railway car, as shown in Fig. 1. Since the amount of heat to be transferred to improve conditions in the railway car, or for other duties, was unknown, the apparatus was designed to transfer about 1000 B.t.u. per hr. The temperature difference across each coil was assumed to be 10° F. or an over-all temperature gradient of 20° F. plus the difference between the temperatures of the liquid on the hot and that on cold coils necessary to produce circulation. The heat transfer coefficient K was taken as 2 for the evaporator and 5 for the condenser in the ice bunker. The surface areas of the coils as constructed were as follows: evaporator, 58 sq. ft.; condenser, 18 sq. ft. Both coils had an over-all vertical

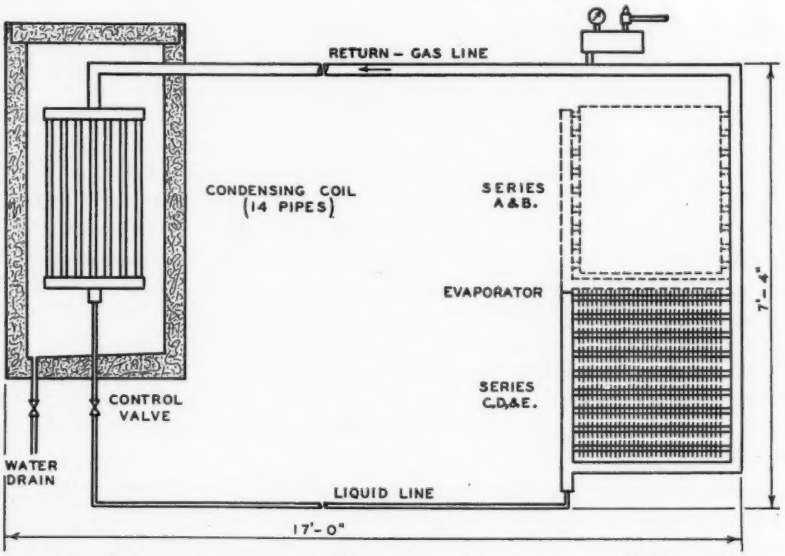

FIG. 1. *Diagram of apparatus showing positions of the evaporator in different series.*

height of 36 in. The condenser was made up of vertical pipes welded into horizontal manifolds, while the evaporator was made up of horizontal finned pipes welded into vertical manifolds.

Further details of construction, and the general experimental arrangement, are evident from Fig. 1. The coils were connected by $\frac{3}{8}$-in. pipe below the liquid level, and by 2-in. pipe above the liquid level. The condenser coil was placed in an insulated, water-tight bunker which was filled with ordinary water ice of about the same size as that used in railway cars. The evaporator coil was hung in a large room at about 80° F. since this gave an over-all temperature difference of about 50° F. between the hot and cold regions. Suitable equipment was provided for determining the liquid level, the total pressure, the temperature of the room, and that of the several heat exchange surfaces. A valve was also provided in the small "liquid" line to stop circulation during "blank" tests.

The method of test consisted of weighing the amount of ice melted, by draining the bunker over known time intervals. An individual test usually extended over a 48- to 72-hr. period, the amount of ice melted being determined over 6- to 8-hr. intervals, and the charge of ice tamped or supplemented as necessary. Other observations were made more frequently. Blank tests were first conducted with the circulation shut off to determine the heat leakage through the bunker and other parts of the system. A similar test was then made with the ammonia in circulation and the net heat transfer of the system was determined by difference. The amount of ice melted by heat leakage yielded remarkably similar results throughout the course of the tests.

Results

The results of these investigations are reported in Fig. 2 and Table I. In the first two series of experiments the condenser and evaporator coils were on the same level, and the effect of the ice level in the bunker, and the liquid ammonia level in the system, were determined under these conditions. It is evident from both the figure and the table that the quantity of heat transferred was rather low. Fig. 2 shows that the capacity of the system did not decrease until the ice level fell to a point about 12 in. below the top of the condenser. Heat conduction along the vertical pipes in the condenser may explain why a decrease in capacity did not occur at an earlier stage.

TABLE I

Series of experiments	Number of experiments per series	Position of evaporator in relation to condenser	Liquid level in 36-in. evaporator and 36-in. condenser		Average height of ice on 36-in. condenser in ice bunker	Average room temp., °F.	Average evap. temp., °F.	Net heat transferred by the system, B.t.u.
			Condenser	Evaporator				
A	4	Vertical and at same level	18 in.	18 in.	24 in. or more to covering	65	60	220
B	4	Vertical and at same level	9 in.	9 in.	24 in. or more to covering	63	56	320
C	5	Vertical and below cond.	Empty	Full	24 in. or more to covering	80	61	840
D	6	Vertical and below cond.	Empty	Full	Covered with ice	78	—	865
E	2	Vertical and below cond.	Empty	Full	Covered with ice and water	85.5	55.5	2900

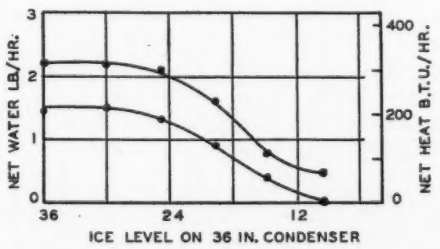

FIG. 2. *Effect of ice level around the condenser on heat transfer (Series A, lower curve; Series B, upper).*

When both coils are on the same level, the effective heat transfer surface of each coil is affected by the liquid level. In the condenser the space occupied by the liquid is not available for condensing the gaseous phase, while the majority of the heat transfer in the evaporator takes place below the liquid level. In Series *A* both coils were half-filled, and in Series *B*, one-quarter filled with liquid. Table I shows that the heat transfer was greater in *B*. Since this occurred under conditions that decreased the capacity of the evaporator coil, but increased the capacity of the condenser, it must be concluded that the condenser coil was the factor limiting the capacity of the

entire system. Although it is desirable to maintain the cooling surface at the highest possible level in the loading space, it is evident from the above that the evaporator must be below the level of the condenser in order to obtain the full capacity of both coils.

The evaporator coil was then lowered to a point below the condenser coil, and the system charged with liquid ammonia to fill the evaporator coil only. This arrangement permitted both coils to operate at full capacity. Comparison of the net heat transfer in Series *B* and *C* shows that this arrangement increased the heat capacity about 500 B.t.u. per hr., part of which may be attributed to an increase in room temperature.

In the experiments reported under Series *D*, the finned evaporator was enclosed in a vertical duct constructed from insulating board. This arrangement facilitated a downward movement of the warm air from the higher levels and should tend to offset the disadvantages of lowering the cooling coil. The results showed that the addition of this duct had no effect on the capacity of the system, the amount of heat transferred being essentially the same as in Series *C*. A number of thermocouples placed in the duct above and below the evaporator showed that the air was cooled 11.3° F. in one passage over the coil, and that the volume of air circulated was about 70 cu. ft. per min.

In Series *C* and *D* it was observed that the surface temperature of the evaporator varied by as much as 10° F. from time to time, and the net heat transferred in different experiments under the same conditions varied from 650 to 1040 B.t.u. per hr. These variations were finally traced to the condition of the ice in the bunker, heat transfer being greatest for a short period after tamping the ice, and then gradually diminishing to the lower values which prevailed over the greater part of the experimental period. This behaviour indicated that the capacity might be increased by improving the conditions for heat transfer between the coil and the ice in the bunker. It was evident that this could be accomplished by allowing the water from the melting ice to accumulate in the bunker.

The final series of experiments was conducted in this way. Experimentally it proved simpler to fill the bunker with a known quantity of ice and add water at 32° F. at the start of an experiment and determine the quantity of each remaining at the end of the test. The net heat transfer in these experiments was 2900 B.t.u. per hr. as shown in Table I. Other measurements showed that the mean temperature of the ice and water mixture was 37° F., that the temperature of the air leaving the evaporator was 18° F. lower than that of the air entering the duct, and that approximately 150 cu. ft. per min. of air was circulated over the evaporator.

These results were obtained with a prevailing temperature difference, between the cooling medium and the air surrounding the evaporator, of about 50° F. As mentioned before, this is apparently the condition prevailing in end-bunker railway cars under certain conditions. In order to be of value the temperature in the warm region should be reduced about 20° F., a con-

dition that would reduce the over-all temperature difference to about 30° F. Assuming a linear relation between heat transferred and temperature difference, the results obtained in Series *E* indicate that a capacity of about 1750 B.t.u. per hr. would be obtained with the present system over a temperature difference of 30° F. In designing the system for about 1000 B.t.u. per hr., an over-all temperature gradient of 20° F. was assumed, and the above results indicate a capacity of 1150 B.t.u. per hr. under these conditions.

In these experiments no provision was made for measuring the actual liquid temperature within the condenser and evaporator. However, the surface temperature of the liquid line leaving the bunker and the surface temperature of the evaporator were essentially the same within the error of measurement under the conditions prevailing in these tests. From this and the observed performance, it appears that the necessary circulation pressure was provided by a relatively negligible difference in the temperature of the liquids in the hot and cold coils. Other observations indicate that the differences between the temperature of the liquid in the condenser and that of the ice and water in the ice bunker, and between that of the liquid in the evaporator and the mean effective air temperature, were approximately equal.

Discussion

The results show that a system of this sort is capable of transferring useful quantities of heat over relatively small temperature differences without the use of forced circulation. In order to avoid the need for an excessively large condensing coil where water ice is used, it is necessary to allow the water to accumulate in the bunker to improve heat transfer from the coil. When solid carbon dioxide is used the difference between the temperature of this solid and that of the coil is so much greater that the addition of a non-freezing liquid to facilitate heat transfer may be unnecessary.

A system of this sort may be found useful for the control of temperature, or for reducing spatial variations in temperature in applications where sources of power are lacking. The reduction in spatial temperature variations is accomplished by the use of coils that can be suitably located and distributed throughout the space. The value of such a system for improving conditions in end-bunker railway cars depends on a number of other factors which have not been studied and need not be discussed here.

When dry ice is used for cooling, excessively low temperatures may result, with consequent waste of refrigerant and product deterioration, unless some means of temperature control is provided. Water ice will seldom produce detrimentally low temperatures but may result in temperatures lower than can be economically maintained under certain conditions, e.g., rural egg grading and packing stations. The use of the present indirect system would permit the ice to be placed in a well insulated bunker to reduce wastage and also permit thermostatic control of the temperature in the space.

Thermostatic control would consist of a sensitive element acting on a suitable closure member in either the liquid or gas line, to modulate or stop the circulation. In this connection the maximum pressure to be overcome need only be that of the head of liquid in the condenser. Observations made during the course of the present experiments indicate that the temperature in the gas line is essentially that of the surrounding space. This suggests placing the sensitive element in the gas line. The closure member should also be placed in the gas line since the excess pressure developed in the evaporator would return the liquid already in the evaporator to the condenser through the liquid line. Closing the liquid line would permit appreciable heat transfer following closure, since the liquid in the evaporator would have to distil over into the condenser coil with consequent absorption of heat.

Acknowledgments

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STUDIES ON HOMOGENEOUS FIRST ORDER GAS REACTIONS

XI. THE DECOMPOSITION OF BENZYLIDENE DIACETATE, *o*-CHLOROBENZYLIDENE DIACETATE AND BENZYLIDENE DIBUTYRATE[1]

BY N. A. D. PARLEE[2], J. C. ARNELL[3], AND C. C. COFFIN[4]

Abstract

Benzylidene diacetate, *o*-chlorobenzylidene diacetate, and benzylidene dibutyrate decompose unimolecularly at rates given by the equation previously found for crotonylidene diacetate and furfurylidene diacetate, viz., $k_1 = 1.3 \times 10^{11} e^{\frac{-33000}{RT}}$. The fact that these esters all have a double bond at the same distance from the breaking point of the molecule is considered significant in connection with their identical reaction velocity, which is about six times that of ethylidene diacetate. Benzylidene diacetate decomposes at the same rate in both the liquid and vapour states to reach an equilibrium given by the equation $\ln K = 13.3 - \frac{10100}{RT}$. The reverse reaction with a rate given by $k_2 = 5.8 \times 10^7 e^{\frac{-22900}{RT}}$ is characterized by a steric factor of 10^{-4}.

Introduction

Previous work (6) has shown that the decomposition velocity of ethylidene diacetate homologues is increased by the presence of a double bond in the aldehyde part of the molecule. Thus, at any temperature, crotonylidene diacetate decomposes more than four times as fast as butylidene diacetate. Moreover, the fact that crotonylidene diacetate and furfurylidene diacetate, which are alike in having a double bond at the same distance from the breaking point of the molecule, react at exactly the same rate suggests that unsaturation has a pronounced effect on the stability of these compounds. The present paper deals with the decomposition of three other esters having a double bond in the same position, viz., benzylidene diacetate, *o*-chlorobenzylidene diacetate, and benzylidene dibutyrate. It may be stated at once that as far as reaction rate and activation energy are concerned, these esters are indistinguishable from the crotonylidene and furfurylidene diacetates.

As the esters of this series can be divided into a slow and a fast group—the latter being characterized by the presence of a double bond—a special effort

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Contribution from the Laboratory of Physical Chemistry, Dalhousie University, Halifax, N.S. Part X of this series appeared in the Journal of Chemical Physics, 7 : 315. 1939.

[2] *Graduate student in chemistry and holder of a Bursary (1936–37) under the National Research Council of Canada. Present address: Dominion Steel and Coal Company, Sydney, N.S.*

[3] *Graduate student in chemistry and holder of a Bursary under the National Research Council of Canada (1939–40).*

[4] *Associate Professor of Chemistry.*

was made to decide definitely whether the rate differences are due to differences in the A or the E of the Arrhenius equation. Ethylidene diacetate and benzylidene diacetate were chosen as representative examples of each group and were carefully studied over a wide temperature range. It is concluded that the evidence is in favour of the previously advanced hypothesis (3) that E remains constant throughout the series.

The benzylidene diacetate decomposition, being particularly clean cut and reproducible, was studied in both the liquid and gaseous states. Equilibrium constants of the liquid system ester $\rightleftharpoons$ aldehyde + anhydride were also determined over a wide range of temperature, and the velocity of the reverse reaction was calculated.

Experimental

The esters were prepared by refluxing the aldehydes (freshly distilled in an atmosphere of carbon dioxide) with an excess of acetic anhydride containing a trace of fuming sulphuric acid as catalyst. They were purified, after neutralizing the catalyst with sodium acetate, by fractional vacuum distillation and by recrystallization. In the case of benzylidene diacetate, several independent preparations were used for the velocity measurements.

The vapour phase reactions were followed in an improved apparatus of the same type as that already described (2). The main improvement consisted in the addition of a device (S, Fig. 1) to introduce the ester after the mercury surface at C had attained the temperature of the reaction chamber B. A glass rod cemented into the ground glass stopper S and shaped as shown in the figure holds the bulb of reactant in the recess K when the mercury is raised to

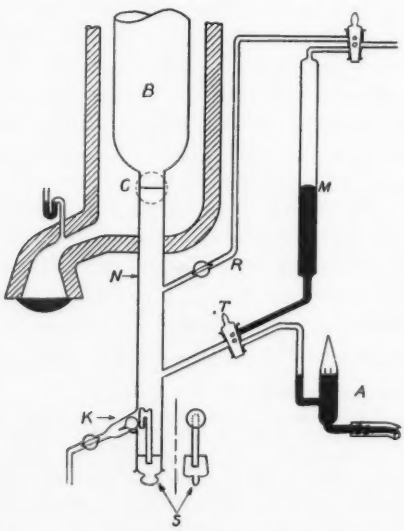

FIG. 1. *Apparatus for vapour phase reactions.*

the window *C*. After the mercury column has reached thermal equilibrium with its surroundings, the stopper *S* is turned through 90°. The released bulb immediately shoots up through the mercury and explodes in *B*. The use of the by-pass *R* for evacuation keeps the mercury in *M* from being fouled with reaction products. The tube *N* is cleaned with cotton swabs after each run.

The liquid phase decompositions were carried out in small sealed tubes hung in vapour thermostats (nitrobenzene, 211°; bromobenzene, 157°) provided with a pressure regulator (4). Runs as long as 100 hr. have been made in this apparatus without any temperature fluctuations being visible on a 0.1° thermometer hanging in the vapour. After a known time in the thermostat the bulbs were removed and their contents analysed for acetic anhydride with 0.1 *N* baryta.

Results

The results of the vapour phase decompositions are given in Table I. The velocity constants (Column 3) were calculated either by the Guggenheim method (8) or by plotting $\log \frac{P_0}{2P_c - P}$ against time (2). In Fig. 2 the negative logarithms of the velocity constants (Column 4) are plotted against the reciprocals of the absolute temperatures (Column 5). The initial pressures, which varied between 2 and 40 cm. without affecting the reaction rate, are not listed.

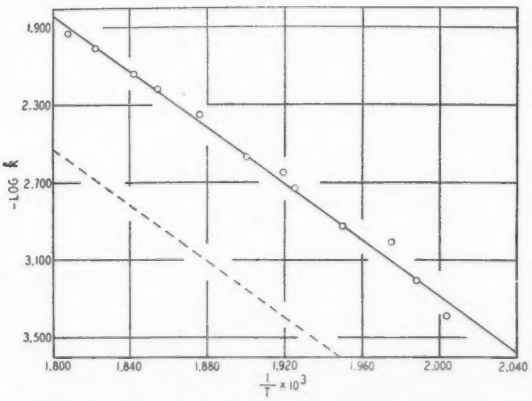

FIG. 2. — *log k plotted against 1/T.* ○, *Benzylidene esters. Broken line, ethylidene diacetate. (The lines are drawn at an activation energy of 33,000 cal.)*

As before (5), the straight line through the points of Fig. 2 has arbitrarily been given a slope corresponding to an activation energy of 33,000 cal. It is evident that this fits the data within the probable errors of experiment. For purposes of comparison the data for ethylidene diacetate (2) (broken line) have been included in the diagram. The rate equation ($k = 1.3 \times 10^{11} e^{\frac{-33000}{RT}}$)

TABLE I

REACTION VELOCITIES IN THE VAPOUR PHASE

Run No.	T, °abs.	$k \times 10^4$ (sec.$^{-1}$)	$-\log k$	$1/T \times 10^3$
Benzylidene diacetate				
22	553.0	110		
23	553.2	123		
24	553.0	119	1.929	1.808
25	553.1	119		
12	548.7	91.1		
13	548.7	93.1		
14	548.7	90.2	2.040	1.822
15	548.7	91.1		
26	543.0	76.1		
27	542.9	67.0		
28	542.9	70.1	2.139	1.842
29	543.0	71.8		
30	543.0	77.4		
6	539.3	67.2		
7	539.3	57.6		
8	539.3	59.0		
9	539.3	57.5	2.220	1.854
10	539.3	63.1		
11	539.3	57.6		
31	533.0	48.4		
32	533.0	43.2	2.355	1.876
33	533.1	40.8		
1	526.4	26.9		
2	526.4	27.4		
3	526.4	27.0	2.569	1.900
4	526.4	27.7		
5	526.4	26.2		
34	523.0	21.9		
35	523.0	22.4	2.637	1.912
36	523.0	23.6		
37	523.0	24.4		
16	519.4	17.1		
17	519.4	18.7	2.733	1.925
18	519.4	19.2		
19	519.4	19.2		
48	515.2	26.0		
49	515.2	13.0	2.678	1.939
50	515.2	25.0		
38	513.0	12.2		
39	513.0	11.5	2.924	1.949
40	513.0	11.9		
41	513.0	12.1		
20	506.3	8.20		
21	506.3	8.84	3.071	1.975
42	502.9	6.16		
43	503.0	6.55	3.201	1.988
44	502.9	6.16		

TABLE I—*Concluded*

REACTION VELOCITIES IN THE VAPOUR PHASE—*Concluded*

Run No.	T, °abs.	$k \times 10^4$ (sec.$^{-1}$)	$-\log k$	$1/T \times 10^3$
Benzylidene diacetate—Concluded				
45	492.9	3.14		
46	493.0	4.73	3.390	2.029
47	492.9	4.35		
Benzylidene dibutyrate				
23	553.0	94.0		
26	553.1	87.4	1.991	1.808
27	552.9	123		
16	542.9	58.1		
18	543.5	67.8		
19	543.0	60.7	2.202	1.842
24	542.9	62.8		
25	542.9	64.4		
1	533.0	41.2		
13	533.0	38.0		
14	533.0	38.4	2.412	1.876
15	533.0	37.4		
5	523.0	22.2		
6	523.1	22.6		
7	523.0	24.6	2.636	1.912
30	523.0	22.3		
31	523.0	23.7		
10	513.0	14.0		
11	513.0	14.5		
12	513.0	14.6	2.851	1.949
28	513.0	14.1		
29	513.0	13.4		
8	503.1	7.25		
21	503.0	6.76	3.151	1.988
22	503.0	7.20		
o-Chlorobenzylidene diacetate				
18	553.0	116		
19	553.0	77.5	2.007	1.808
20	553.0	102		
12	543.0	57.2		
13	543.0	64.0	2.205	1.842
14	543.0	66.0		
9	533.0	28.2		
10	532.8	31.6	2.538	1.876
11	533.0	27.2		
16	523.0	18.9		
17	523.0	18.1	2.733	1.912
6	513.0	10.9		
7	513.0	10.6	2.966	1.949
8	513.0	11.0		
4	503.0	6.17		
5	503.0	6.59	3.184	1.988
15	503.0	6.92		

already given (6) for the furfurylidene and crotonylidene diacetate fits the benzylidene esters equally well.

It is of obvious importance to decide definitely whether these relatively small differences in reaction rate (e.g., between ethylidene diacetate and benzylidene diacetate) are due to differences in activation energy, or whether they arise from a connection between the structure of the molecule and the A factor of the rate equation. A difference in E of only 5000 cal., for example, is sufficient to account for the difference between the specific reaction rates of ethylidene diacetate and benzylidene diacetate. An attempt was therefore made to settle this question by obtaining as precise data as possible over a wide temperature range on highly purified samples of representatives of the "fast" and "slow" groups of esters. Benzylidene diacetate was chosen as typical of the former group and ethylidene diacetate of the latter. Alternate runs were made with these esters by several different operators in different pieces of apparatus. Special attention was paid to temperature measurement and control. The benzylidene diacetate results are included in the data of Table I. The ethylidene diacetate results agreed with the equation already published (2) (broken line in Fig. 2) and are not quoted here. It is concluded from the data at hand that there is no appreciable difference between the activation energies of the fast and slow esters, and that the rate differences are due to variations in the temperature independent factor with molecular structure.

It is of interest to compare the rates of clean-cut reactions in the liquid and vapour states. The benzylidene diacetate decomposition was therefore studied in the liquid phase at 211 and 157°C. It was found to lead to a well defined equilibrium and to be very susceptible to traces of catalytic impurities, especially at the lower temperature where the purely thermal reaction is not very fast ($k = 2.4 \times 10^{-6}$ at 157°). From the fact, however, that several very carefully treated lots of bulbs (drawn from new tubing cleaned with cotton plugs and live steam and finally flamed) gave decompositions within a few per cent of the values calculated from the vapour phase data and corrected for the reverse reaction, it is believed that the rates in each phase would be the same if catalytic impurities could be entirely eliminated from the liquid. It has already been shown (9) that the chlorinated esters decompose at the same rate in the liquid and gaseous states. The velocity of this series of reactions has thus been found to be the same in the liquid phase as it is in the gas at pressures down to 0.1 mm. mercury (7)—a concentration range of about 10^5.

By adding a trace of sulphuric acid to the ester it was possible to determine the equilibrium constants of the liquid system benzylidene diacetate $\rightleftarrows$ benzaldehyde + acetic anhydride over a wide temperature range. The amount of decomposition, which is very rapid in the presence of the acid, was determined by plunging the bulbs containing the equilibrium mixture into carbon dioxide and ether, and analysing for anhydride. The results are summarized in Table II.

TABLE II

THE EQUILIBRIUM: $K = \frac{\text{CONC. BENZALDEHYDE} \times \text{CONC. ACETIC ANHYDRIDE}}{\text{CONC. BENZYLIDENE DIACETATE}}$

Temp., °abs.	% Decomposition	K	Log K
484.2	85.6	16.3	1.212
430.1	65.7	4.23	0.626
373.1	32.7	0.611	$\bar{1}$.786
293.1	6.3	0.020	$\bar{2}$.301

The values of log K plotted against $1/T$ fall fairly well on a straight line the slope of which corresponds to a heat of reaction of 10,100 cal. Two direct determinations of the heat of the reaction (ΔH) between the anhydride and aldehyde in a large specially built ice calorimeter (broken during the third run) gave 9,950 and 10,200 cal. per mole of ester formed. The activation energy of the presumably bimolecular association is thus probably about 33,000 − 10,100 = 22,900 cal. The equilibrium constant at any temperature is given by the equation $\ln K = 13.3 - \frac{10100}{RT}$.

Assuming that the equilibrium constant is the same in both phases, it is possible to calculate the velocity of the bimolecular gaseous association from the equations $k_2 = k_1/K$ and $E_2 = E_1 - H$. This leads to the expression $k_2 = 5.8 \times 10^7 e^{\frac{-22900}{RT}}$ litre-mole^{-1} sec.$^{-1}$ for the rate of reaction between benzaldehyde and acetic anhydride. Since the collision frequency at concentrations of 1 mole per litre is of the order 10^{11} litre-mole^{-1} sec.$^{-1}$, the steric factor of the association must be about 10^{-4} i.e., only one collision in 10^4 results in reaction. The only fairly clean-cut gaseous bimolecular associations involving complex molecules that have so far been studied are about half a dozen examples of the diene synthesis (for summary see reference 1), which are also characterized by steric factors of the order of magnitude 10^{-4} to 10^{-5}. It is suggested by Benford and Wassermann (1) that this rarity of fruitful collisions is due to the circumstance that two colliding molecules may have to make simultaneous contact at two different and specified points before reaction can occur. It is interesting that the aldehyde-anhydride association, in which the orientation requirements ought to be much less exacting than in the diene syntheses, should have a steric factor of the same order of magnitude.

The decomposition of these benzylidene esters resembles very closely that of other members of this series. The substitution of butyrate for acetate groups and of chlorine for hydrogen makes no apparent difference in either reaction rate or its temperature coefficient. The activation energy (33,000 cal.) is that characteristic of the series. The A factor of the Arrhenius equation, while being 76 times as great as that of methylene diacetate, the slowest member of the series, is still unusually low (10^{11} sec.$^{-1}$) for unimolecular reactions, and is the same as that previously found for crotonylidene and fur-

furylidene diacetates. It is perhaps significant that all these "fast" esters have a double bond equidistant from the seat of reaction. Attempts are being made to prepare and study esters having double bonds farther removed from the breaking point of the molecule.

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LINOLENIC ACID AND ITS ISOMERS[1]

By J. W. McCutcheon[2]

Abstract

The preparation of linolenic acid by a modification of Rollett's method is described. The melting point of the solid hexabromide is placed at 181.9° C. The boiling point, specific gravity, iodine value, and refractive index of the ethyl ester, and the melting point of the free acid, were determined. A soft white gummy hexabromide of melting point 145° to 150° C. was isolated from the ethyl ether soluble hexabromides in proportions approaching that obtained for the solid isomer. Reduction of the so-called liquid hexabromostearic acid gave a product that could not be differentiated from the solid derivative. These facts support the general theory regarding the isomerism noted in the bromo derivatives of the non-conjugated unsaturated fatty acids, and indicate that the so-called α- and β-linolenic acids are identical. No attempt is made to assign any particular *cis-trans* configuration to this acid, nor to exclude the theoretical existence of seven others.

In a previous report by the author (5) on the isomerism of linoleic acid, it was concluded that the isomerization noted during bromination occurs in the bromo derivatives only, owing to the asymmetric carbon atoms produced. The reduced bromides regenerate the same parent acid. This conclusion has been confirmed recently by the work of Riemenschneider, Wheeler, and Sando (6) and also by the work of Kass and Burr (3). The latter demonstrate that *cis-trans* isomerism of linoleic acid can be accomplished and that a new solid tetrabromide of m.p. 79° C. is thereby formed. Theoretical considerations would lead to the belief that linolenic acid would behave in a similar manner, and it was considered expedient at this time to investigate the isomerism of this acid from the above point of view.

The investigation described herein had for its object: *(a)* the establishment of additional constants of the esters of the solid hexabromo derivatives; *(b)* the possible separation of the bromo isomers; and *(c)* the preparation of the liquid derivative in a state pure enough for its comparison with the solid.

Experimental Data

Raw Material

Fresh linseed oil of guaranteed purity* was used. The analysis is given in Table I.

Preparation of the Fatty Acids

In the preparation of the fatty acids the method recommended by the American Oil Chemists' Society (1) was adopted. Linolenic acid was prepared

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Contribution from the Department of Chemistry, University of Toronto, Toronto, Ont. From a thesis presented in partial fulfilment of the requirements for the Ph.D. degree at the University of Toronto.

[2] *Superintendent, Lightfoot Schultz, Co., Hoboken, N.J., U.S.A. Formerly Chief Chemist, Lever Brothers, Limited, Toronto, Ont.*

* *Kindly supplied by the Dominion Linseed Oil Co. Ltd., Baden, Ont.*

TABLE I

ANALYSIS OF THE LINSEED OIL USED

Free fatty acid (as oleic), %	0.83	Analysis of the fatty acids, %	
Colour	12.0 red/50 yellow		
Iodine value (Wijs)	181.3	Saturated acids (Twitchell)	9.5
Saponification value	198.1	Oleic acid (by calc.)	15.4
Total fatty acids, %	95.6	Linoleic acid (by calc.)	29.8
Unsaponifiable, %	0.6	Linolenic acid (by calc.)	44.7
		Unsaponifiable	0.6

essentially according to the method of Rollett (7), with the following modifications: (i) ethyl ether was used as a solvent rather than glacial acetic acid; (ii) a special purification process for the solid hexabromide was introduced; (iii) debromination and esterification were carried out as two separate steps; (iv) the esterification was completed in the absence of zinc.

The Bromination

Seventy grams of linseed fatty acids was dissolved in 1800 ml. of ethyl ether and cooled to 10 to 20° C. With vigorous stirring and keeping the temperature below 20° C., liquid bromine was run in from a dropping funnel until a permanent red colour was produced, and then a fair excess added. Thirty-five millilitres of bromine was required. The whole was allowed to stand overnight at 3 to 5° C., after which the precipitated hexabromides were removed on a Büchner and washed several times with fresh ethyl ether. The yield of solid hexabromide was approximately 21 to 23% of the weight of fatty acids used, representing 8% linolenic acid. This is consistent with the results obtained with linoleic acid if it is taken into consideration that only 25% of the total hexabromides formed came down as the solid isomer.

Purification of the Solid Hexabromide

A careful study was made of this problem so as to establish the purity of the product by means of melting points. It was early discovered that unlike the tetrabromostearic acid, the hexabromo compound could not be melted into the capillary tubes, since a slight decomposition took place which lowered the melting point two, three, and even five degrees. Subsequently, all melting points were obtained by the closed tube method. The usual solvent for recrystallizing hexabromostearic acid has been glacial acetic acid, or better, benzene. However, the solubility in these liquids is poor and they are difficult to handle. It was found that, of a great many solvents tried, 1 : 4 dioxane offered the best possibilities, and a number of experiments were carried out that established the following points: (i) the solubility gradient between warm and cold dioxane is so great that little advantage is gained in using mixed solvents; (ii) unlike the solvent mixture used for tetrabromostearic acid, dioxane solution supersaturates readily and the maximum precipitation is not attained until the solution has stood several hours or preferably overnight;

(iii) the dioxane causes no decomposition of the hexabromide itself since solutions have been kept for months with no discoloration or changes in melting point of the recovered bromide. Owing to heat sensitivity, solutions of pure hexabromide will discolour slightly on being held for some time near the boiling point of the dioxane; (iv) samples crystallized clear from benzene gave a slightly murky solution with dioxane; this indicated the presence of an impurity, soluble in the former solvent but insoluble in the latter. Filtering and recrystallizing from dioxane gave clear solutions, indicating that the insoluble material did not form progressively with handling, but was probably formed during bromination. This was confirmed by the following experiment:—

Linseed oil fatty acids (677 gm.) dissolved in 4500 ml. of ethyl ether were partially brominated with 100 ml. of liquid bromine and allowed to stand three months. At the end of this time the bromination was complete. The ethyl ether insoluble product contained 32%, or 34.2 gm. of material insoluble in 1 : 4 dioxane. The remainder (68%) had the melting point of hexabromostearic acid; this indicated it to be a pure normal compound. The insoluble material was recrystallized by dissolving it in a small amount of glacial acetic acid and precipitating from a 50% mixture of 95% ethyl alcohol and chloroform. The snow-white product had a melting point of 178.5° C. and contained 66.7% bromine (theoretical for septabromostearic acid, 66.9%). It is interesting to note that this substitution product was formed in the absence of excess bromine, through prolonged standing only. The above results indicate an additional factor in favour of dioxane as a solvent for the recrystallization of the hexabromide, although under normal brominations these dioxane insoluble septabromides are formed in minute traces only, and do not appreciably affect the melting point of the hexabromide.

The results obtained by using both solvents are given in Table II. The closed capillary tube method was used, with a special thermometer 9 in. long, range 167 to 195° C., graduated in 0.1° C. It was checked against a U.S. Bureau of Standards thermometer at 167.5°, 180.4° and 190.5° C. No differences could be noted. Stem corrections were avoided by using the thermometer completely immersed.

TABLE II

MELTING POINT OF HEXABROMOSTEARIC ACID

Well washed crude hexabromide, ppt'd. from ethyl ether only	181.4° C.
Crude sample recrystallized from benzene	181.9° C.
Above sample twice recrystallized from dioxane	181.9° C.
Crude recrystallized three times from dioxane	182.0° C.
Above recrystallized from benzene	181.9° C.

From the above data the melting point of solid hexabromostearic acid is placed at 181.9° C. (corr.). Bromine content, 63.18% (theory, 63.28%).

The final procedure adopted for recrystallizing the hexabromide is as follows:— 100 gm. of hexabromide is added to 600 ml. of 1 : 4 dioxane and

warmed to 70 to 80° C. to effect solution. The solution is filtered if cloudy, then allowed to stand overnight at 15° to 20° C., after which the precipitated crystals are filtered off by suction, and washed thoroughly with ethyl ether by transferring to a separate beaker and refiltering. The product falls from the Büchner as a fine white powder. Yields are approximately 80% of theory.

Solubility of the Hexabromide in 1 : 4-Dioxane

Experiments indicate a solubility of 25 to 28 gm. per 100 ml. of solvent at 70 to 80° C.; 5 gm. per 100 ml. still remain in solution even after several hours standing at 15° C., but this drops to approximately 3 gm. per 100 ml. on standing overnight.

Debromination and Esterification

These operations were carried out in a manner identical with that described for linoleic acid (5), except that the time of refluxing in the presence of zinc was extended from two to six hours, and in the absence of zinc from one to three hours. The ethyl ester distils at constant temperature without the formation of any residue, and the acidity, as linolenic, has been found to vary from batch to batch from 0.20 to 0.40%. If the free fatty acid content approaches 1%, a very definite rise in distillation temperature of 1° to 2° C. will be noted. In such cases, or in any case where the product was to be used for the determination of physical constants, the free acids were removed as follows:—

The ester was thoroughly shaken in a separatory funnel with about three times its volume of ½% sodium carbonate solution at a temperature of not over 50° C. The resulting emulsion was broken by centrifuging at 2200 r.p.m. (relative centrifugal force of about 1000× gravity) in 100-ml. tubes, and the alkaline solution pipetted off. Warm water washes were carried out in like manner until they were neutral to methyl orange. Usually not more than four washes were necessary. The yield of resulting ester is usually 80 to 85% of theory.

The free acid is prepared just prior to use by the method of cold saponification outlined by Rollett (7). The acid is more unstable than linoleic, samples kept under carbon dioxide for short periods becoming thick and viscous. The acid had a distinct fish odour; this is rather remarkable, since such odours are usually associated with the more highly unsaturated acids of the clupanodonic series. This odour is not noticed in the mixed fatty acids from linseed oil, being probably masked by the natural odour of the oil. The distilled ester had only the normal faint fruity odour similar to that of ethyl linoleate or ethyl oleate. Since it is known that the conversion of ester to acid activates the double bond, it seems likely that the fish odour is generated by such activation and may be caused by the production of polymerized by-product. Typical yields derived from the data of numerous batches are given in Table III.

TABLE III

SUMMARY OF TYPICAL YIELDS—LINSEED OIL TO LINOLENIC ACID

Substance used	Amount, gm.	Substance obtained	Amount, gm.
Linseed oil	400	Fatty acids	348
Fatty acids	348	Hexabromide	80
Hexabromide	80	Recrystallized hexabromide	65
Recrystallized hexabromide	65	Crude ethyl ester	21
Crude ethyl ester	21	Refined and distilled ester	17
Refined and distilled ethyl ester	17	Linolenic acid	12

Boiling Point of the Ethyl Ester

The apparatus and method were identical with those previously described (5). The data obtained are given in Table IV.

TABLE IV

BOILING POINT DATA FOR ETHYL LINOLENATE, TAKEN AT VARIOUS REDUCED PRESSURES

Pressure, mm.	2½	4	6½	11	15	6½
Temp. uncorrected, °C.	173	183	196	209½	216	197
Temp. corrected, °C.	174	184	198	211	218	198½

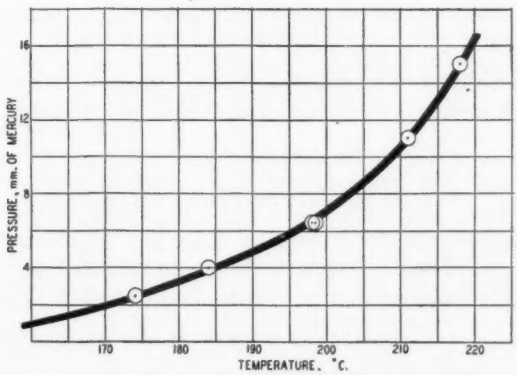

FIG 1.. *Boiling point curve of ethyl linolenate.*

Specific Gravity of the Ethyl Ester

This was determined by means of a 10 ml., Gay-Lussac type, specific gravity bottle. Data are given in Table V.

TABLE V

SPECIFIC GRAVITY OF ETHYL LINOLENATE

	Temp., °C.	Grams	Specific gravity
Air wt. of pycnometer (*P*)		8.0004	
P + water	15.5	17.9769	Apparent, 0.8958 (15.5°/4° C.)
P + water	25.0	17.9595	True, 0.8959 (15.5°/4° C.)
P + ester	15.5	16.9458 16.9460	Apparent, 0.8889 (25.0°/4° C.)
P + ester	25.0	16.8776 16.8772	True, 0.8890 (25.0°/4° C.)

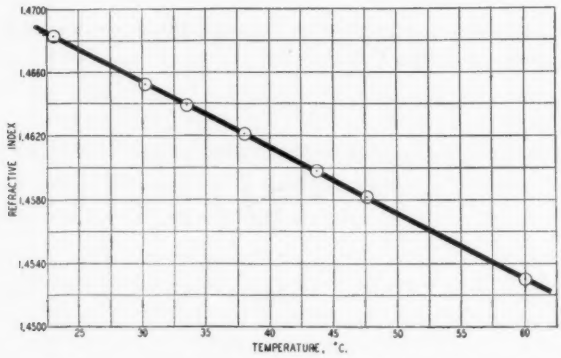

FIG. 2. *Refractometer readings for ethyl linolenate at various temperatures.*

Refractive Index of Ethyl Linolenate

Values were determined by means of an Abbé refractometer, using sodium light.

TABLE VI

REFRACTIVE INDEX OF ETHYL LINOLENATE

Temperature, °C.	23.0	30.2	33.6	38.0	43.7	47.6	60.0
Butyro-reading (calc.)	63.7	58.9	56.9	54.2	50.7	48.3	40.9
Refractive index	1.4683	1.4652	1.4639	1.4621	1.4598	1.4582	1.4530

Iodine Value

The iodine value of the ester was determined by the method of Wijs (1).

Methyl linolenate, 257.33 (theoretical, 260.57)
Ethyl linolenate, 245.48 (theoretical, 248.64)

Melting Point of Linolenic Acid

Melting point values, using a special low temperature thermometer $+10°$ to $-50°$ C., were $-16\frac{1}{4}$ to $-17°$ C.

Separation of the Bromo Derivative of Linolenic Acid

Linolenic acid (5.0227 gm.) was brominated in the usual manner in 200 ml. of petroleum ether. The white gummy precipitate (7.5993 gm.) was extracted with ethyl ether, thereby leaving a white insoluble residue (*A*) of 2.2510 gm., identified later by melting point as the normal hexabromide. The ethyl ether extract deposited a small amount (1 gm.) of white gum (*B*) on chilling to $-60°$ C. The remainder, evaporated to dryness, gave 3.65 gm. of yellow syrup (*C*).

The original petroleum ether filtrate was chilled to $-60°$ C., whereupon it deposited a small amount of sticky gum (*D*). Evaporation to dryness left 0.383 gm. of white non-sticky gum (*E*).

The above products were tested in a large number of solvents and the following experiment carried out. Linolenic acid (13.5113 gm.) was dissolved in 500 ml. of ethyl ether and brominated in the usual manner at 0 to $-5°$ C. The precipitated hexabromide (*F*) of m.p. (recrystallized) 181.9° C. weighed 7.07 gm., representing 19.21% of the theoretical quantity of bromides formed. The precipitate gave only the merest trace of dioxane insoluble material; this indicated almost complete absence of septabromostearic acid. The solvent was completely evaporated under a stream of carbon dioxide and the residue completely dissolved in 150 ml. of hot isoamyl alcohol. On chilling to 15° C., a heavy white precipitate, which was of a gummy nature, came down. It was separated by centrifuging. This precipitate (*G*) was recrystallized twice from 75 ml. portions of isoamyl alcohol, heating to 75° C. to dissolve, and decanting the centrifuged wash. The vacuum dried sample gave 5.34 gm., or 14.5% of the total theoretical bromides present. Br content, 62.2% (theoretical for hexabromide, 63.28%.) The product was a soft gum melting between 145° and 150° C.

The original isoamyl alcohol filtrate deposited a clear amber oil layer (*H*) after standing several days. This was removed by decantation and the filtrate evaporated completely, first under a stream of carbon dioxide and finally under vacuum. The product (*I*) was pale straw coloured and resembled melted rosin. It closely resembled (*H*) above. Combined (*H*) and (*I*) gave 22.65 gm., or 61.5% of the total bromides formed. Br content, 59.4%.

Linolenic Acid from the Liquid Hexabromides

Linolenic acid (12.60 gm.) was brominated in the usual manner in ethyl ether. The solid hexabromide (2.2 gm.) was filtered off, and the ether evaporated under a stream of carbon dioxide. The resulting mixture of so-called liquid bromides was debrominated and esterified in the usual way. The product (3.1 gm.) was found to have a boiling point of 176° C. at $2\frac{1}{2}$ mm. and a refractive index of 1.4532 at 60° C.

Discussion of Results

Melting Point of the Solid Hexabromide

It is shown that 1:4 dioxane is a more practical solvent than glacial acetic acid or benzene for recrystallizing hexabromostearic acid. In addition, this solvent is superior to benzene in removing at least one type of impurity that may be present, namely, the septabromostearic acid. The melting point, variously reported in the literature (4, 8, 9) as from 178° to 183° C., has been placed at 181.9° C. corrected.

Boiling Point of Ethyl Linolenate

Results show close agreement with the values obtained for ethyl linoleate and indicate the difficulty of trying to separate these compounds by fractional distillation.

The Iodine Value

As for linoleic acid (5), the iodine value by the method of Wijs is approximately 98.8% of theory. The problem whether this is characteristic of the method will be made the subject of further investigation.

Melting Point of the Acid

The only other value noted in the literature (8) places the melting point at −14.5° to −14.4° C.

Isomerism

The theory advanced previously (5) and since supported by the work of Riemenschneider, Wheeler, and Sando (6) and Hilditch and Jasperson (2) would predict the existence of four pairs of enantimorphous hexabromides of linolenic acid. The present investigation has led to the isolation, from the liquid bromide fraction, of one additional hexabromide not previously reported, namely, 14.5% of a white gummy hexabromide soluble in ethyl ether but sparingly soluble in cold isoamyl alcohol. The remainder of the liquid bromide fraction is present in sufficient amount (61.5%) to permit its being a mixture, although definite conclusions on this point cannot be drawn on the data available. The reduction of the mixed liquid bromides to linolenic acid indicates, from the data, that it is not dissimilar to ordinary so-called α-linolenic acid derived from the solid hexabromide. The view advanced previously is thus supported by the data obtained with linolenic acid, and points to the identity of α- and β-acids. By analogy with linoleic acid, we would expect the natural product to be identical also with these acids, a point of view supported by the work of Shinowara and Brown (8).

Acknowledgments

The author wishes to take this opportunity to thank Dr. W. Lash Miller, Professor Emeritus of Physical Chemistry, University of Toronto, and Dr. F. B. Kenrick, Professor of Chemistry, University of Toronto, for their encouragement and advice during the course of the work; to acknowledge the

technical assistance of Mr. E. P. Howarth in obtaining some of the data reported in this paper; and to thank Lever Brothers Limited, Toronto, for their kindness in making available certain pieces of equipment necessary for the work.

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A PHOTOELECTRIC COLORIMETER-FLUORIMETER[1]

By D. K. Froman[2] and W. D. McFarlane[3]

Abstract

A photoelectric colorimeter of the compensating two-photocell type is described. It is simple in design, relatively inexpensive to construct, and satisfactory in performance. By changing the lamp and filters it can be adapted to fluorescence measurements.

Introduction

The most commonly employed methods for determining thiamin (vitamin B_1) are riboflavin (vitamin B_2) involve the measurement of the intensity of the fluorescent light emitted when the solution is irradiated with ultra-violet radiations. In the former case the vitamin is first oxidized to the fluorescent substance, thiochrome. Photoelectric colorimeters are now in general use but it would be an added facility if the instrument were designed so that it could also be employed as a fluorimeter. This is achieved in the instrument described below which is simple in design, relatively inexpensive to construct, and satisfactory in performance.

General Description of the Instrument

Photographs of the instrument are shown in Fig. 1, and in Fig. 2 is shown a horizontal cross-section, at mid-height*. It is essentially a photoelectric colorimeter of the compensating two-photocell type employing two Weston Model 594 matched photronic cells, *K*. In front of one photocell is a standardized test-tube, *X*, containing 5 ml. of solution, and between the cell and a 25-watt filament lamp, *L*, is the appropriate light filter (Fig. 1-E). In front of the other photocell are two polaroid discs, *P*; one of these is fixed and the other can be rotated. A 0° to 90° scale is engraved on *H* opposite a Vernier scale (1/10°) on the top of *G*. A phosphor-bronze cable around *H* and around a shaft allows *H* to be rotated from outside the case. At zero on the scale the polaroids are crossed and at 90° they are parallel. Between the polaroids and the lamp is an adjustable shutter (Fig. 1-C). In operation, as a colorimeter, the shutter is adjusted to give no deflection of the galvanometer when the polaroids are parallel (reading 90°) and the solvent is in the test-tube. The solvent is replaced by the test solution and the back polaroid rotated, towards 0°, until the galvanometer shows no deflection. The galvanometer is a Rubicon "spot light" No. 3402-H, resistance 350 ohms and

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[2] *Assistant Professor of Physics, Department of Physics, McGill University, Montreal, Que. Formerly Assistant Professor of Physics, Macdonald College.*

[3] *Professor of Chemistry, Macdonald College.*

* *Detailed specifications with blue-print of Fig. 2 may be obtained by writing to the authors.*

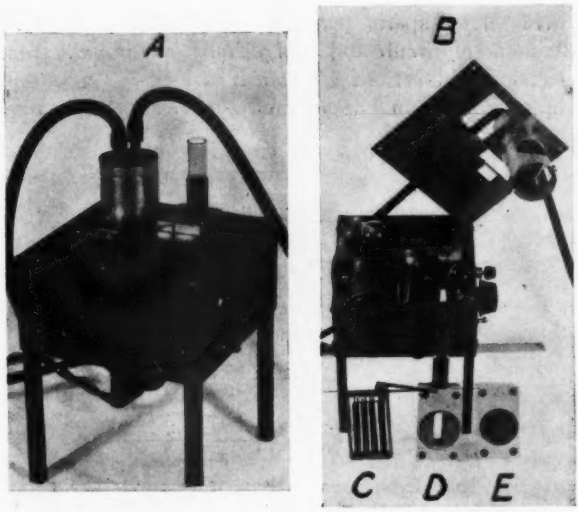

FIG. 1. *Photographs of instrument.* A. *Set up for colorimetry with shutter (C), test-tube holder (D), and light filter holder (E), in position.* B. *Top removed and movable parts set out in front.*

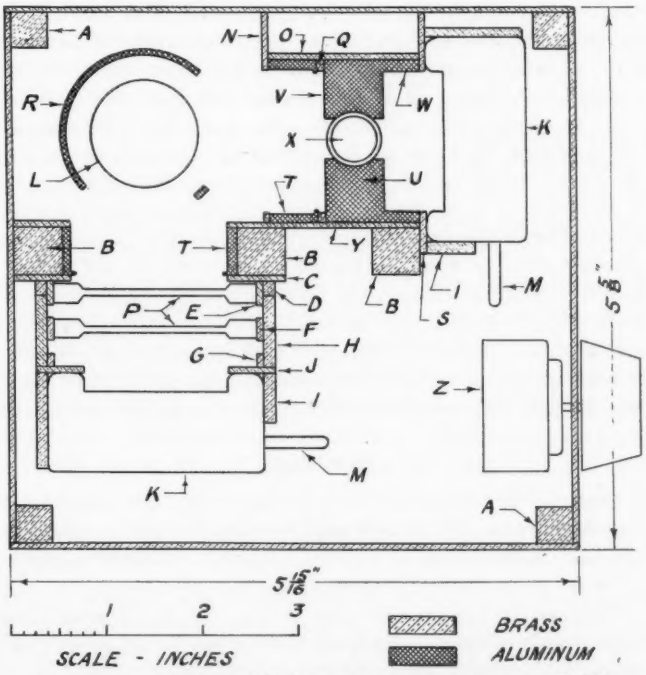

FIG. 2. *Specifications of the instrument—cross-section at midheight.*

sensitivity 0.015 microamperes per mm. The rotation of R through 180° opens the galvanometer circuit and cuts off most of the light from the photocells. The electrical connections are shown in Fig. 3. A water reservoir is set into the top of R and $\frac{1}{4}$-in. copper tubing carries cooling water throughout the box, where space permits.

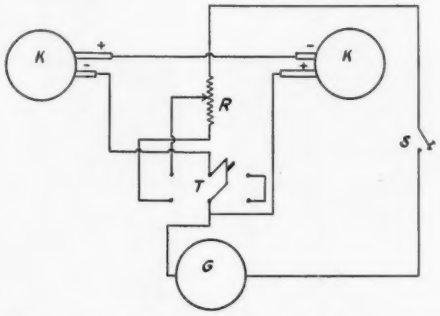

FIG. 3. *Wiring diagram. K, photronic cells; G, galvanometer; S, switch operated by rotating R (Fig. 2); R, 2500 ohm wire wound volume control with knob. (Model H, Ohmite Mfg. Co., Chicago, U.S.A.); T, toggle switch, short neck, double pole, double throw.*

To carry out fluorescence measurements it is necessary only to change the source of light and the light filters. An ultra-violet filter and a straw-yellow Noviol A filter are placed in front of the polaroids with the former adjacent to the lamp. The Noviol A filter removes the ultra-violet radiations but fluoresces a bright yellow. It is this fluorescent light that passes through the polaroids to energize the photocell. On the other side a similar ultra-violet filter is placed in front of the test-tube. Behind the test-tube and in front of the photocell is placed the appropriate light filter, i.e., for thiochrome, a Noviol A to remove ultra-violet radiations and a Corning No. 430 filter to remove the yellow fluorescent light from the Noviol A, but to transmit the blue fluorescent light from the sample; for riboflavin, a Corning No. 351 filter alone to eliminate ultra-violet and interfering blue-green radiations. The intensity of light incident on the two photocells, with solvent in the test-tube, is balanced by a variable resistance with the polaroids crossed (reading 0°). When the solvent is replaced with the fluorescing solution, the polaroids are rotated (towards 90°) until the galvanometer again shows no deflection.

Operation

A. *Colorimetry.* The tungsten lamp is used and cold water is circulated through the instrument. Although water cooling is not so essential as with the mercury arc, nevertheless it is advantageous. A filter* suitable for the particular colour is put into the block T on the side next to U. The other block T is replaced by the shutter (Fig. 1) whose opening is adjustable from the top of the block. The polaroids are set parallel (reading 90°) and a test-

* *Rubicon Company, 29 North Sixth St., Philadelphia, U.S.A.*

tube* containing 5 ml. of the solvent only is put into *U*. (A number of test-tubes were compared and those matching perfectly were retained for use.) The tube *R* is rotated to connect the galvanometer, and the shutter is adjusted to give no deflection. The test solution is then put in *U*, the back polaroid is rotated to give no deflection of the galvanometer and the angular scale on *G* is read. The concentration of chromogen is then found from a calibration curve or by calculation (see below).

B. *Fluorimetry.* A General Electric H3–T10 mercury clear lamp is used and is operated with transformer No. 59G-1A. An ultra-violet filter† and a fluorescing glass†† are placed in the filter block *T* in front of the polaroids, with the ultra-violet filter adjacent to the lamp. A similar ultra-violet filter is placed in the filter block *T* on the other side and the appropriate light filters are placed behind the test-tube in block *U*. For thiochrome, a Noviol A and Corning No. 430 are used, with the former adjacent to the test-tube; for riboflavin, a Corning No. 351 is used. Water is circulated through the instrument, a test-tube containing the solvent is placed in *X*, the lamp is turned on, and the variable resistance is switched in and adjusted (with the polaroids set at zero) until the galvanometer shows no deflection. It is found that the light intensity on the polaroids is too great to balance the current with the variable resistance. This difficulty is overcome by covering the face of the ultra-violet filter with 70-mesh copper gauze. The gauze is trimmed down until an approximate adjustment of the galvanometer can be obtained without the resistance switched in. The gauze is held rigidly in position by the split-steel ring which holds the filters in place. It requires about 10 to 15 min. for the lamp to heat up, and it is found that reproducible results are best obtained by keeping *R* rotated, to connect the galvanometer. The fluorescence of the Noviol filter depends on the temperature and in this way an equilibrium is obtained. The ultra-violet filter may crack, however, but after being cemented together it will not crack again.

A number of test-tubes containing a solution of quinine sulphate are matched and those giving identical readings are retained for use and are marked so that they can be placed in the instrument again in the same position. After adjusting the galvanometer to zero with the solvent in the test-tube, the test solution is substituted for the solvent and the polaroid rotated until the deflection of the galvanometer is again zero. The angular scale is read and the concentration of fluorescing substance determined by reference to a calibration curve, previously prepared, or by calculation (see below). Where excessive fluctuations in line voltage prevail, the blank reading should be checked frequently.

* *Standardized test-tube No. 3788-C (125 × 14 mm. O.D.) and graduated at 5 ml. Arthur H. Thomas Co., Philadelphia, U.S.A.*

† *Corning red purple corex No. 986—polished disc 1¼ in. diameter and 5 mm. thick.*

†† *Corning, straw yellow, Noviol, Shade A, No. 038, 1¼ in. diameter and 2 mm. thick.*

Theory and Performance

The use of two matched photocells opposing each other is of considerable advantage in balancing out fluctuations in light intensity. It is inconvenient to have to make, for each coloured or fluorescent solution, a detailed calibration of the instrument with standard solutions in order to determine an unknown concentration. For solutions that show an absorption coefficient proportional to the concentration of solute, an observation on a single known concentration is sufficient to calibrate the colorimeter if the ratio of the light intensities transmitted by the two solutions is known. For this reason it is valuable to investigate, for the instrument described here, how the transmitted light intensity varies with the angle between the polarization planes of the two polaroid discs.

The polaroid discs do not transmit completely polarized light, partly because all the light is not incident normally upon them. Hence the relative intensity of the light transmitted by the two polaroids will not be accurately proportional to the square of the cosine of the angle between their polarization planes. Let θ represent the reading on the angular scale of the instrument. Then θ is the complement of the angle between their polarizing planes. Let α be the ratio of the intensity of the light transmitted when the polaroids are crossed to the intensity when they are parallel. We assume that this fraction, α, of the light intensity is not affected by relative rotation of the polaroids, but that the remainder of the light follows the ordinary laws, well known for the transmission by Nichols' prisms. Then if I be the intensity transmitted at angle θ, and I_0 the intensity transmitted when $\theta = 90°$,

$$I = I_0[\alpha + (1 - \alpha) \sin^2 \theta]. \tag{1}$$

The assumption that α is not a function of θ can be tested by applying Equation (1) to a series of known solutions of a substance which obeys Beer's law, viz.:

$$I = I_0 10^{-kc}, \tag{2}$$

where I_0 is the intensity transmitted by the pure solvent, I is transmitted by a concentration c, and k is a constant involving the extinction coefficient and the geometry of the absorber.

By using the colorimeter with balanced cells described here the I and I_0 of Equations (1) and (2) are made equal. Whence, solving for KC,

$$KC = -\log_{10}[\alpha + (1 - \alpha) \sin^2 \theta]. \tag{3}$$

Once the value of α is determined for the instrument, and the value of k is determined by using a single sample of known concentration, any concentration can be determined. α can be determined either by means of an auxiliary experiment in which the intensities of light are measured, or by using a series of known concentrations, each pair of which can be used to find a value for α. It is found that both methods give the same value for α.

Using a value of 0.090 for α, as determined by means of an auxiliary experiment, values of K have been determined for a number of different coloured solutions of known concentrations. The probable error of a single observation

for K includes the error in dilution and chemical procedure involved in preparing the coloured solution, the error in the applicability of Equation (3), and random errors in setting and reading the instrument. These random errors, estimated from the reproducibility of readings, amount to about 1.2% over the useful range of the instrument. For example, the value of K was found to be constant within a probable error of a single observation of 3.4% for 12 concentrations of xanthophyll varying from 0.15 to 3.0 γ per ml., and giving polaroid readings varying from about 20° to 75°. Similarly, the value of K was found constant within a probable error of 1.8% for 12 concentrations of iron dipyridyl containing from 0.24 to 3.64 γ of iron per ml.

When the instrument is used as a fluorimeter, the average intensity of the ultra-violet light throughout the fluorescing solution depends on the concentration of the solute. If, however, the solution is sufficiently dilute, the ultra-violet absorption of the fluorescent compound can be neglected. In this case the intensity of the fluorescent light is proportional to the concentration. Since balance is obtained with a blank when the polaroids are crossed, α can be dropped from Equation (1), whence the concentration, C, is given by

$$C = K' \sin^2 \theta \qquad (4)$$

where K' is a constant for the instrument and fluorescent substance.

Values of K' have been determined for quinine sulphate, thiamine, and riboflavin. As in the case of colorimetry, random instrumental errors estimated from reproducibility data are about 1.2%. For 11 concentrations of quinine sulphate ranging from 0.16 to 1.36 γ per ml., K' was found constant with a probable error in a single observation of 2.9%. For 12 concentrations of thiamine ranging from 0.15 to 0.55 γ per ml., K' was constant within 5.9%, and, for eight concentrations of riboflavin ranging from 0.1 to 4 γ per ml., K' was constant within 6%.

The approximate theory given above [See Equations (3) and (4)] holds most accurately in the range of polaroid readings from 20° to 70°. The use of an empirical calibration curve is essential for accurate work outside this range. Even near the middle of the range, empirical calibration will render higher accuracy than the equations but it is much more tedious. Readings can be made, but with decreasing precision, down to concentrations of the order of 1/10 of the lowest values quoted above.

The total cost of the material and equipment required to build the instrument, including the galvanometer and light filters, is about $145.00.

Acknowledgment

The fluorescence measurements were kindly made by Mr. F. P. Griffin.

MICROCHEMICAL TECHNIQUE

IV. THE MICRODETERMINATION OF MERCURY AND HALOGEN IN ORGANOMERCURIC HALIDES[1]

By Gladys O. Stonestreet[2] and George F. Wright[3]

Abstract

A procedure has been outlined for simultaneous volumetric determination of chlorine or bromine and mercury in organomercurials. Attention is called to some modifications of the Zacherl and Krainick method which was adapted to this purpose.

The difficulties involved in the volumetric determination of mercury in presence of halogen have hindered the investigation of organomercurials because the acetoxymercuri-compounds, which are usually the initial products of such studies, are frequently less stable and more difficult to purify by crystallization than are the chloromercuri and bromomercuri analogues to which they are so easily converted (14, p. 361). Since the volumetric method, involving decomposition with nitric acid in a sealed tube with subsequent thiocyanate titration (8), is not applicable to organomercurials which contain halogen, they have been analysed for mercury by precipitation as the sulphide, by electrodeposition (10, p. 145), or by other reductions (2; 5; 6, p. 686; 11). None of these methods have been very successful on the microscale. Therefore it seemed worth while to devise a volumetric procedure which would provide for the microvolumetric determination of halogen and mercury in the same sample.

Since mercuric monobasic salts are volatile, nitric acid is unsatisfactory as a decomposition agent except in a sealed tube. The method of Zacherl and Krainick (16) involving dichromate in sulphuric acid as oxidizing agent seemed applicable, since mercuric sulphate, being nonvolatile, would remain behind while the halogen was evolved. The method has not been applied to the determination of iodine, but this is of little consequence in organomercurial studies, because the iodomercuri-compounds are so unstable that they are not, by choice, satisfactory derivatives. The method was found to work admirably; the halogen from representative organomercuric halides was determined with an accuracy of 2%. It was found necessary, however, to introduce certain variations. In particular, first, a uniform gas flow was required. Since this was difficult to maintain when the oxygen was passed through a gas-washing bottle containing sodium carbonate (which also acted as a bubble counter), the oxygen was measured by means of a flowmeter (13) from a gas holder containing 5% sodium hydroxide. Second, the original directions specify a "Messerspitze" of silver dichromate (1) and potassium dichromate

[1] *Manuscript received March 5, 1940.*
Contribution from the Chemical Laboratory, University of Toronto, Toronto, Ont.
[2] *Fourth year honours student in Chemistry.*
[3] *Associate Professor of Chemistry.*

mixture, and this indefinite specification has been interpreted by Niederl (9, p. 134) as 0.5 gm. although 0.05 gm. is the correct amount. It was found more convenient to use, instead of the prescribed reagents, an equal volume of a solution prepared by heating sulphuric acid with potassium dichromate and silver dichromate until the salts dissolved. Third, since organomercuric halides always contain a low percentage of halogen, the final titration must be very precise. Since the indicator seemed to be oxidized during the final conversion of hypochlorous to hydrochloric acid by means of hydrogen peroxide, it was found that high results would be obtained (owing to destruction of the indicator) unless the methyl red was added after the conversion was complete.

The satisfactory results thus obtained for halogen indicated that the whole of the mercury had been retained in the digestion vessel, *D*, of the Zacherl and Krainick apparatus. It was, however, contaminated with silver and potassium dichromate. It was found that dichromate could be removed by reduction with sodium arsenite, and the silver with hydrochloric acid, but the presence of chloride ion made the separation unsuitable for thiocyanate titration. A method reported by Fischer (3, 4) which depended on the conversion of the green-coloured solution of diphenylthiocarbazone (dithizone) to an orange complex in the presence of mercuric ion was not affected by chloride ion and seemed applicable. Winkler (15) used the method for determination of small amounts of mercury in leafy vegetables. He titrated his analytical sample and a mercury standard simultaneously against a solution of dithizone in carbon tetrachloride. With suitable modifications, his procedure worked quite satisfactorily on an aliquot of the sulphuric acid residue containing the mercury of the sample. These modifications include, first, preparation of a standard solution of mercuric sulphate containing the same amounts of potassium dichromate, silver dichromate, and sulphuric acid which are encountered in the sulphuric acid residue from the halogen determination. Second, this standard must be treated with sodium arsenite and hydrochloric acid in the same manner as the sample. Finally, in order to cancel any errors in colour comparison and accuracy of volumetric equipment, a check determination was always run, reversing the pairs of burettes, pipettes, and separatory funnels used simultaneously for sample and standard. The check results, which varied 0.2 to 0.6%, were then averaged. Using these precautions, results averaging 75 parts per 10,000 (0.75% error) were obtained.

Analyses of representative organomercuric halides are shown in Table I. Only in the case of the styrylmercuric bromide was difficulty encountered, but here the trouble is significant, because it will undoubtedly be encountered with other mercurials. The results for mercury were 3 to 10% low when decomposition was accomplished by the half-hour heating period in the dichromate-sulphuric acid solution according to the Zacherl and Krainick procedure for halogen. The values for halogen, on the other hand, were good. This indicated that decomposition of the C–Hg bond was incomplete; accordingly, when the halogen determination was completed, 0.5 cc. of fuming nitric acid (sp. gr., 1.52) and 0.5 cc. of 20% fuming sulphuric acid were added

TABLE I

ANALYSES OF ORGANOMERCURIC HALIDES

Compound	Halogen				Mercury		
	Weight, mg.	% Halogen, calc.	% Halogen, obs.	% Error	% Mercury, calc.	% Mercury, obs.	% Error
Phenylmercuric chloride	4.772	11.32	11.55	2.0	64.04	63.91	0.2
	4.721	11.32	11.52	1.8	64.04	63.87	0.3
	6.125	11.32	11.54	1.9	64.04	64.25	0.3
1,2-Diphenyl-2-methoxy-ethylmercuric chloride	3.788	7.94	8.00	0.8	44.78	44.20	1.3
	6.076	7.94	7.83	1.4	44.78	44.72	1.2
5-Chloromercuri-2-furfuryl acetate	5.601	9.48	9.41	0.7	53.47	53.48	0.1
	4.574	9.48	9.57	1.0	53.47	53.23	0.5
	5.463	9.48	9.28	2.1	53.47	52.77	1.3
	5.766	9.48	9.35	1.4	53.47	53.43	0.1
2-Methoxycyclohexyl-mercuric chloride	4.686	10.15	10.24	0.6	57.45	56.94	0.9
	6.119	10.15	10.36	2.0	57.45	57.80	0.6
	5.488	10.15	10.42	2.6	57.45	56.94	0.9
5-Chloromercurifurfural	5.830	10.74	10.72	0.2	60.59	61.05	0.8
	4.354	10.74	10.79	0.5	60.59	59.66	1.5
3-Chloromercuributanol-2	5.711	11.47	11.70	2.0	64.88	64.67	0.3
	4.579	11.47	11.53	0.5	64.88	65.11	0.4
2-Phenyl-2-methoxyethyl-mercuric bromide	6.034	19.23	19.61	1.9	48.27	47.40	1.8
	4.760	19.23	19.15	0.4	48.27	47.88	0.8
Styrylmercuric bromide	6.295	20.83	20.63	1.0	52.29	52.93	1.2
	5.668	20.83	20.59	1.2	52.29	52.48	0.4
Average % error				1.3			0.8

and the mixture was heated gradually to 140° over a 20 min. period to simulate the decomposition method of Rupp (7, p. 2769; 12). This modification required that 1 cc. of 1.5% urea solution be added to both standard and diluted sample, probably to remove nitrite, which is known to interfere with the end-point (15).

The method outlined above thus serves for determination of halogen and mercury in the same sample and since the ratios of these elements are 1 : 1 in the halomercuri-linkage, the greater information to be gained by analysis of the organo-mercuric halides than of the corresponding acetates makes the former derivatives now a preferred choice.

Procedure

A 4 to 6 mg. sample of mercurial is weighed into the decomposition flask, *D*, of the Zacherl and Krainick apparatus, and the apparatus is assembled with the receiver, *G*, containing 5 to 5.5 ml. of 0.02 *N* sodium hydroxide and 1 ml. of 30% hydrogen peroxide (Merck). Through the dropping funnel, *B*,

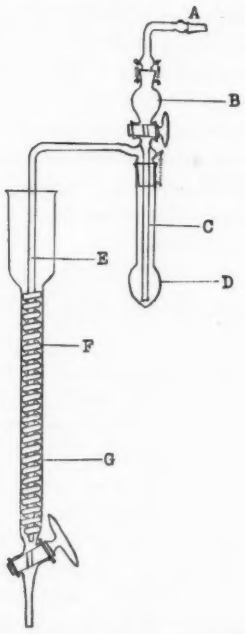

FIG. 1. *Apparatus for the determination of halogen.*

is added 2 ml. of a solution containing 0.30 gm. of potassium dichromate and 0.30 gm. of silver dichromate per 10 ml. of concentrated sulphuric acid (sp. gr., 1.84). The oxygen supply from a gas holder containing 5% sodium hydroxide as the confining liquid is carefully regulated by means of a flowmeter to 8 ml. per min. After the customary heating period of 30 min. at 115 to 125° C., the contents of the receiver, *G*, are drained into a quartz flask. The washing of the receiver is carried out economically so that the final volume does not exceed 20 ml. This is treated with a calculated excess of 0.02 *N* hydrochloric acid and boiled for 30 sec. To this hot solution, free of oxidizing agent, is added 0.002 ml. of alkaline methyl red solution (9, p. 54) and the titration completed with the 0.02 *N* alkali.

Calculation: log cc. (alkali − acid − perhydrol blank) + log normality of alkali + log factor + log 100 − log weight of sample; antilog of whole = % halogen.

Factors: chlorine, 35.457; bromine, 79.916.

The sulphuric acid solution remaining in the reaction vessel, *D*, is washed into a 500 ml. volumetric flask. Ten millilitres of this 500 ml. volume is transferred to a 200 ml. separatory funnel to which is also added 1 cc. of a 5% sodium arsenite solution. When the solution has become colourless, 60 ml. of water is added, followed by 4 ml. of concentrated hydrochloric acid.

Into a similar 200 ml. separatory funnel is pipetted 10 ml. of a standard solution of mercuric sulphate prepared in the following manner: A solution of 0.750 gm. of mercuric sulphate (c.p. Baker's Analysed) in 6 ml. of concentrated sulphuric acid was diluted to 1 litre. Six millilitres of this concentrated standard solution was treated with 2 ml. of the oxidizing solution used by Zacherl and Krainick, and the whole then diluted to 500 ml. to make the dilute, working standard solution. The 10 ml. portion of this solution was treated with sodium arsenite, water, and hydrochloric acid in the same manner as for the solution to be analysed.

A preliminary titration should be carried out first. Two 25-ml. burettes, each containing a carbon tetrachloride solution of dithizone* are placed above two separatory funnels.

Ten millilitres from one burette is added to the aliquot of sample solution, the funnel closed, and shaken exactly 40 times in 10 sec. This standardized shaking is important, since the end-point varies with time of contact. While the layers in the first funnel are separating, an identical addition and agitation is carried out on the standard solution in the second separatory funnel. The orange-coloured layer in the first funnel is drawn off, 1 ml. of dithizone solution added, and the funnel shaken according to the prescribed mode. Subsequently, 1 ml. portions are added alternately to standard and sample solutions until the orange of the mercury complex is no longer formed, but the green of the reagent persists after shaking. This change marks the end-point. It is advisable to record a burette reading after each addition of dithizone reagent.

For the exact titration, 10 ml. aliquots of standard and sample solutions are prepared for titration in exactly the same manner as described above. To each is added 2 ml. less than the total amount of dithizone solutions required in the preliminary titration. The next addition is 1 ml. to each, then 0.2 ml. portions until a slight green colour appears. The titration is completed with 0.1 ml. portions until a colour comparable with that of the titrating solution is obtained. The exact titration is now repeated, burettes, pipettes, and separatory funnels having been reversed from sample to standard solution. The two results are averaged.

Calculation: log cc. dithizone used by sample + log 0.750 + log 6 + log factor + log 100 − log cc. dithizone used by standard − log weight of sample; antilog of whole = % mercury.

$$\text{Factor: } = \frac{\text{Hg}}{\text{HgSO}_4} = 0.6761.$$

** The dithizone solution was prepared according to Winkler (15). One gram of diphenylthiocarbazone (Eastman Kodak, No. 3092) in 50 ml. of chloroform was extracted three times with 100-ml. portions of 1% aqueous ammonia. The combined aqueous layers were acidified with 1 ml. of concentrated nitric acid and extracted with 50 ml. of chloroform. This solution was evaporated on the steam bath and the residue dried at 48° C. and 10 mm. A concentrated solution of the substance, which solution should not be used if over a month old, is prepared by dissolving 0.05 gm. in technical carbon tetrachloride to make a volume of 100 ml. Five millilitres of this solution is diluted to 200 ml. with carbon tetrachloride to make the titrating solution, which must be used on the day that it is prepared.*

In the event that results for mercury are not reproducible, after the halogen determination is completed, 0.5 ml. of 20% fuming sulphuric acid (Mallinckrodt) and 0.5 ml. of fuming nitric acid (sp. gr., 1.52, Mallinckrodt) are added. The decomposition flask, *D*, is gradually heated to 140° C. over a 20 min. period while the gas flow of 8 ml. per min. is maintained, the receiver, *G*, having been filled with enough water to cover the tip of the inlet tube, *E*. Subsequent operations are identical, except that 1 ml. of a 1.5% aqueous solution of urea is added to sample and standard in the separatory funnels prior to titration.

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A SMALL-SCALE ELECTRODIALYSIS CELL[1]

By H. B. Collier[2]

Abstract

An electrodialysis cell for removing electrolytes from small volumes of colloidal solutions is described. On dialysis of a solution half-saturated with ammonium sulphate, only 2% of the salt remained after three hours.

The electrodialysis cell herein described is a modification of that proposed by King (1) and was designed for the removal of electrolytes from small volumes (5 to 25 ml.) of protein solutions. The anode and cathode are platinum and mercury respectively, and water circulates through both an inner and an outer compartment, separated by cellophane membranes from the solution in the middle compartment. The large surface permits rapid dialysis and at the same time provides efficient cooling. It was found that a solution of haemoglobin, coagulating at about 60° C. on direct heating, was not denatured in the cell until the current exceeded 50 ma.

Very rapid dialysis of half-saturated ammonium sulphate solution was achieved, using running tap water, as indicated in Table I. Estimations were made colorimetrically by the Nessler method. The solution increased about 50% in volume and was neutral at the end of the run. The rate of dialysis is about three times that obtained by Taylor, Parpart, and Ballentine (2) with their "Rapid Circulating Dialyser".

TABLE I

Electrodialysis of half-saturated ammonium sulphate

Time, hr.	Current, ma.	Voltage	Concentration	
			Molar	%
0	50	10	2.32	100
0.5	50		1.17	50
1.0	50		0.56	24
1.5	49		0.28	12
2.0	48		0.115	6.7
2.5	47	50	0.074	3.2
3.0	47		0.037	1.6
4.0	46		0.0093	0.4
5.0	37	100	0.0013	0.06

Description of the Apparatus

The construction of the cell is indicated diagrammatically in Fig. 1. The cell is made of Pyrex glass, with a standard taper 29/26 ground joint, *G*. The water connections are as indicated in the diagram. The glass head is double, with two flanges, *F*, to which are tied the cellophane sacs, *H*. The sacs are

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[2] *Research Associate.*

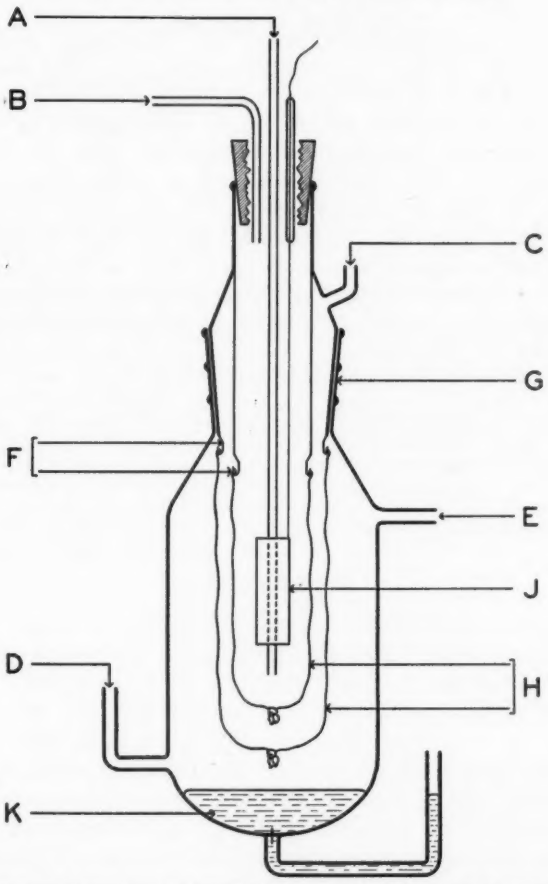

FIG. 1. *Diagram of small-scale electrodialysis cell.* A. *Water inlet of inner compartment.* B. *Water outlet of inner compartment.* C. *Inlet tube for middle compartment.* D. *Water inlet of outer compartment.* E. *Water outlet of outer compartment.* F. *Flanges of glass head.* G. *Ground joint.* H. *Cellophane sacs.* J. *Platinum anode.* K. *Mercury cathode.*

of cellophane tubing, 18 and 27 mm. in diameter, knotted at the lower end. The capacity of the middle compartment may be varied somewhat by altering the lengths of the sacs.

The anode is of platinum foil, 1 × 2 in., bent to form a cylinder surrounding the water inlet tube, *A*. The cathode is a pool of mercury, *K*, contact being made by means of a platinum wire sealed through the bottom of the vessel.

It is probable that the middle and inner compartments could be made completely separable by the use of another small ground joint. With a Pyrex adapter (bushing type), standard taper 29/42–19, and a male joint, standard taper 19/38, the cellophane sacs would be attached to flanges on the lower ends of the adapter and the male joint.

Method of Dialysis

The cellophane having been tested for leaks, the glass head is inserted into the cell. *A* is connected to the water supply, *B* and *D* are connected by a rubber tube, and *E* leads to the drain. The solution to be dialysed is poured into the middle compartment through the inlet, *C*. The electrodes are connected to the d-c. line, in series with a milliammeter and a suitable resistance (about 4000 ohms for 200 volts). Samples may be removed during dialysis by means of a small rubber tube (No. 10 catheter) inserted into the middle compartment through the inlet, *C*. Tap water is suitable for the removal of high concentrations of salt employed in the salting out of proteins.

The apparatus was made according to specifications by Ingram and Bell, Ltd., Montreal.

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THE PROBLEM OF PLASTEIN FORMATION

I. THE FORMATION OF A PLASTEIN BY PAPAIN[1]

By H. B. Collier[2]

Abstract

Papain, activated by cyanide, cysteine, or hydrogen sulphide, produces a plastein, a protein-like substance, from concentrated peptic or papain digests of egg albumin. This activity is suppressed by boiling, aeration, the addition of copper salts, hydrogen peroxide, iodoacetate, or alloxan, indicating that free SH groups are essential. The optimum pH for plastein formation is 4.8; that for hydrolysis of the plastein by papain is about pH 4.2. It is concluded that concentration alone controls the direction of the enzyme action, the optimum pH and oxidation-reduction conditions being practically identical for both formation and hydrolysis of plastein.

The rate of plastein formation varies with substrate concentration, above a minimum value. With constant substrate concentration, the rate of plastein formation varies as the square-root of the enzyme concentration. Hydroxylamine reduces the activity of the papain by about one-half. A similar effect is produced by treatment with phenylhydrazine, followed by benzaldehyde. Phenylhydrazine alone has no effect, whereas benzaldehyde alone depresses the activity very strongly.

Introduction

It has been found that the addition of the enzyme papain to concentrated protein digests results in the formation of a precipitate which has the properties of a plastein. Kurajeff (16) in 1901 first noted a "coagulation" of peptone solutions upon the addition of papain, although some of his observations do not agree with those to be reported.

Wasteneys and Borsook (22) have summarized the evidence in support of their thesis that peptic plastein is a protein and its formation a true enzymatic synthesis. Other workers, e.g., Alcock (1), are of the opinion that the protein nature of plastein has not been conclusively established. The present paper is limited to a description of the conditions affecting the formation of a plastein by papain: the nature of the plasteins will be discussed in a following paper. Bergmann and co-workers (2–9) have recently studied in detail the hydrolytic properties of papain. They have also reported (10–12) a synthetic action of this enzyme and have demonstrated that the physico-chemical conditions for synthesis are identical with those for hydrolysis.

The present paper describes the formation of a plastein, by papain, from concentrated peptic or papain digests of egg albumin. It is demonstrated that this plastein formation depends upon the presence of active enzyme, which in turn requires free sulphydryl groups. Various factors influencing this action of papain are described.

[1] *Manuscript received March 20, 1940.*
Contribution from the Department of Biochemistry, University of Toronto, Toronto, Ont.

[2] *At the time, holder of a personal grant from the Banting Research Foundation. At present, Research Associate, Institute of Parasitology, McGill University, Macdonald College, Que.*

Experimental

Preparation of Materials

A solution of commercial egg albumin was coagulated, the coagulum washed repeatedly, and digested by commercial pepsin or papain to the point where trichloracetic acid at 2.5% concentration gave no further precipitate. The digest was then adjusted to pH 4.7, boiled for one hour, filtered, and evaporated to a concentration of 60 to 70 mg. of total nitrogen per ml. These concentrated hydrolysates were used as substrates for plastein formation.

The papain employed for plastein formation was partially purified by extraction of commercial papain (B.D.H.) with water, and thrice repeated precipitation with ethanol at a concentration of 55% by volume. The product was a soluble powder containing about 11% total nitrogen, giving a weak Molisch reaction, and a slight nitroprusside test which was intensified by potassium cyanide.

Papain supposedly free from natural activator was also prepared by the method of Grassmann (15), threefold precipitation being employed. This preparation gave a strong direct nitroprusside test, and was found to be fully active without addition of activators.

Analytical Methods

Enzyme and activator were dissolved in citrate buffer of pH 5.0 and allowed to stand overnight in the refrigerator. This solution, together with the substrate, was placed in the water bath at 38° C. one-half hour before the beginning of the experiment, and the two were mixed at zero time. The formation of plastein was indicated by the appearance of a white precipitate or of an opaque jelly.

One-millilitre samples were pipetted from the mixture at suitable intervals, diluted and boiled immediately, cooled and made up to 25 ml. Determinations of total and non-plastein nitrogen were made upon aliquots by micro-Kjeldahl, the non-plastein nitrogen being estimated on the filtrate obtained 30 min. after the addition of trichloracetic acid to 2.5% final concentration. Since in many cases it was found impossible to pipette uniform samples, because of the viscosity of the mixture, the results from the several samples of one series were all corrected to the same total nitrogen content as the initial sample.

The analytical figures as given represent averages of repeated determinations agreeing within less than 1%. In each case the appropriate blank values for nitrogen content of enzyme, activator, or inhibitor have been deducted, the results thus expressing net plastein formation.

Time Course of Plastein Formation

Wasteneys and Borsook (23) showed that the formation of plastein by pepsin proceeded rapidly for the first few days, but about 12 days were required to reach equilibrium. Table I shows that the formation of plastein by papain follows approximately the same time course, 10 to 16 days being required to reach equilibrium, under the conditions of the experiment. In

TABLE I

FORMATION OF PLASTEIN BY PAPAIN IN RELATION TO TIME INTERVAL

(The figures refer to milligrams of nitrogen per millilitre, and plastein nitrogen as percentage of total nitrogen.)

Time, days	*A*				*B*			
	Total N, mg.	N.P.N., mg.	Plastein N		Total N, mg.	N.P.N., mg.	Plastein N	
			Mg.	%			Mg.	%
0	59.3	59.3	0	0	59.8	59.8	0	0
1	59.3	55.5	3.8	6.4	59.8	58.3	1.5	2.5
2	59.3	54.5	4.8	8.1	59.8	58.3	1.5	2.5
3	59.3	55.8	3.5	5.9	59.8	58.0	1.8	3.0
5	59.3	54.2	4.8	8.1	59.8	55.8	4.0	6.7
10	59.3	49.5	9.8	16.5	59.8	52.7	7.1	11.9
16	59.3	49.5	9.8	16.5	59.8	51.3	8.5	14.2
23	59.3	49.5	9.8	16.5	59.8	51.2	8.6	14.4
27	–	–	–	–	59.8	51.2	8.6	14.4

Experiment A, 300 mg. of papain, activated with 225 mg. of cysteine hydrochloride, was added to 21 ml. of papain digest; in Experiment B, 150 mg. of papain, activated with 112 mg. of cysteine hydrochloride, acted upon the same volume of digest, both being carried out at 38° C.

Since only relative results were required in the study of papain to be described, the time interval was in most cases limited to two to three days.

Properties of the Plastein

The plastein formed by papain was soluble in strong hydrochloric acid and in dilute sodium hydroxide. It gave a purple biuret reaction, a positive ninhydrin test, and a positive Molisch reaction. The direct nitroprusside test was negative, but became strongly positive after addition of cyanide.

A washed and dried sample contained 12.6% total nitrogen, of which 9.0% was found to be free amino nitrogen. The corresponding values reported for peptic plastein by Wasteneys and Borsook (22) are 13.6 and 8.4% respectively.

Activation of the Enzyme

The purified papain was able to form plastein, but was completely inactivated by boiling. Treatment of the preparation with hydrogen sulphide, cyanide, or cysteine increased the yield of plastein, and presumably the activity of the enzyme. The following figures give the degree of plastein formation (plastein nitrogen as percentage of total nitrogen) by activated, non-activated, and boiled enzyme in three days at 38°.

(a) Papain activated with hydrogen sulphide	11.8%
(b) Non-activated papain	8.2
(c) Boiled papain	0.5

Grassmann papain was found to be almost fully active, increasing only slightly in activity when treated with cysteine. For example, 60 mg. of

this papain in 6 ml. of substrate gave a yield of 11.8% plastein under certain conditions; the same amount of enzyme, treated with 45 mg. of cysteine hydrochloride, gave 12.6% plastein under the same conditions.

Optimum Hydrogen Ion Concentration

The extent of plastein formation by papain was measured at various hydrogen ion concentrations, the pH being measured electrometrically upon samples diluted 10 times. Fig. 1 gives the results of an experiment covering the range of pH 2–7. Papain (activated with cysteine) in 1.2% concentration was allowed to act for three days at 38° C. upon a papain hydrolysate containing 73.5 mg. of nitrogen per ml. The maximum plastein formation, at pH 4.8, corresponded to 10.7 mg. of nitrogen per ml.

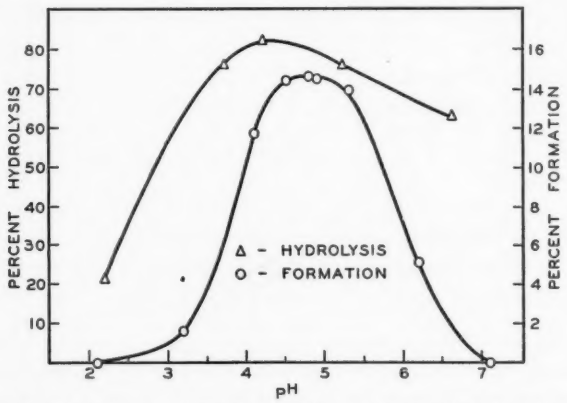

FIG. 1. *Effect of pH upon plastein formation and plastein hydrolysis by papain.*

Papain Hydrolysis of Plastein

In order to compare the optimal hydrogen ion concentrations for formation and for hydrolysis of plastein by papain, the effect of pH upon hydrolysis was studied. Freshly prepared plastein was treated with alkali to destroy enzyme, neutralized and washed repeatedly, and finally suspended in citrate buffer of the desired pH. The substrate was 2% plastein suspended in 0.2 *M* buffer, to which was added papain (activated with hydrogen sulphide) to 0.4% concentration. The degree of hydrolysis was measured after three days at 38°, and the results are indicated in Fig. 1, where 100% corresponds to a hydrolysis of 1.77 mg. plastein nitrogen per ml. Controls with boiled enzyme indicated no significant degree of hydrolysis at any pH.

Optimum Temperature

Since the so-called "optimum temperature" of an enzyme reaction has no absolute significance, the effect of temperature upon the rate of plastein formation was not investigated in detail. The influence of conditions of time and concentration was illustrated by preliminary experiments. In one case, where an unusually concentrated substrate was employed, plastein was formed

with great rapidity at 90° C.; in another experiment *no* plastein was formed at that temperature.

Under the conditions usually employed, with papain–hydrogen-sulphide and papain digest, the following results were obtained after nine hours (plastein nitrogen as percentage of total nitrogen).

38° C.	8.5%	65° C.	15.0%
50	12.4	80	4.9

Effect of Substrate Concentration

To equal volumes (5 ml.) of substrate of varying concentration were added equal amounts of papain (100 mg. papain and 75 mg. cysteine hydrochloride in 2 ml. buffer) and the degree of plastein formation determined after two days at 38°, the resultant values being given in Table II. There appears to be a minimum concentration for plastein formation, under these conditions about 26 mg. of nitrogen per ml. Above this value the extent of plastein formation varies with the substrate concentration.

TABLE II

EFFECT OF SUBSTRATE CONCENTRATION UPON PLASTEIN FORMATION BY PAPAIN

(The figures represent milligrams of nitrogen per millilitre, and plastein nitrogen as percentage of total nitrogen and of non-plastein nitrogen.)

Total N, mg.	N.P.N., mg.	Plastein N		
		Mg.	Per cent of total N	Per cent of N.P.N.
14.1	14.1	0	0	0
27.2	27.0	0.2	0.6	0.6
40.0	36.6	3.4	8.5	9.3
53.1	45.3	7.8	14.7	17.2
67.8	57.1	10.7	15.8	18.8

Effect of Enzyme Concentration

To equal amounts of papain hydrolysate were added varying quantities of papain activated with cysteine, plus sufficient boiled enzyme to give equal concentrations of total enzyme. The results are recorded in Table III, where it is seen that the relation closely follows the square-root law, within the rather limited range of enzyme concentrations employed.

Inhibition of Plastein Formation

It has already been noted that boiling completely destroys the plastein-forming power of papain. The same was found to be true on treatment with a solution of copper sulphate or with hydrogen peroxide. In the former case the enzyme could not be reactivated by subsequent treatment with hydrogen sulphide, but in the latter case a rapid reactivation resulted.

In addition, quantitative tests were made of the ability of various substances to influence the formation of plastein, in order to obtain some idea of the

TABLE III

EFFECT OF ENZYME CONCENTRATION UPON PLASTEIN FORMATION BY PAPAIN

(Enzyme concentrations represent milligrams of dry material per millilitre of mixture. Nitrogen values are given as milligrams per millilitre, and the extent of plastein formation, in three days at 38° C., as percentage of the total nitrogen.)

Active enzyme concentration, mg.	Total N, mg.	N.P.N., mg.	Plastein N		Plastein N ÷ enzyme
			Mg.	%	
0	67.5	67.5	0	0	—
4.3	67.2	61.1	6.1	8.9	2.96
8.6	67.3	58.7	8.6	12.6	2.94
12.8	67.8	56.8	11.0	16.1	3.07
17.1	69.0	57.2	11.8	16.9	2.86

TABLE IV

EFFECT OF INHIBITORS UPON PLASTEIN FORMATION BY PAPAIN

Expt. No.	Treatment	Plastein formation, %
1	*Aeration*—1.25% papain-H_2S in papain hydrolysate; air saturated with water vapour bubbled through for 31 hr.	3.6
	Control, not aerated	11.0
2	*Iodoacetate*—80 mg. papain-H_2S + 62 mg. Na iodoacetate; peptic digest	Nil
	Control without iodoacetate	12.1
3	*Alloxan*—80 mg. papain (non-activated) + 40 mg. alloxan; papain digest	1.3
	Control activated with cysteine, no alloxan	19.3
4*a*	*Hydroxylamine*—80 mg. papain-H_2S + 10 mg. $HONH_2$, one hour at room temp.; peptic digest	9.2
	Control without $HONH_2$	12.1
4*b*	As above, but $HONH_2$ increased to 47 mg.	9.5
	Control	14.4
5	*Phenylhydrazine*—60 mg. Grassmann papain + 120 mg. phenylhydrazine, 14 hr. at 8° and one-half hour at 38°; papain hydrolysate	13.3
	Control without phenylhydrazine	12.1
	Control with phenylhydrazine and boiled enzyme	0.4
6	*Phenylhydrazine and benzaldehyde*—75 mg. Grassmann papain + 125 mg. phenylhydrazine for two hours at 38°, then added 125 mg. benzaldehyde; after another two hours at 38° centrifuged off the phenylhydrazone and tested the activity of the supernatant with papain digest	12.9
	Control, with phenylhydrazine but no benzaldehyde	18.1
7*a*	*Benzaldehyde*—70 mg. papain-H_2S in 7 ml. peptic digest + 2 ml. benzaldehyde; shaken frequently	12.6
	Control with benzaldehyde and boiled enzyme	7.9
	Control with active enzyme and no benzaldehyde	18.1
7*b*	As above, but using papain-cysteine and papain digest	11.6
	Control with benzaldehyde and boiled enzyme	4.7
	Control with active enzyme and no benzaldehyde	22.0

active groups in the enzyme responsible for this property. These experiments followed in general the methods used by Bergmann and co-workers in their study of the hydrolytic action of papain. For the sake of brevity the description of these experiments is summarized in Table IV. In each case, unless otherwise stated, the enzyme and test substance were dissolved in 2 ml. of citrate buffer at pH 5.0, allowed to stand one hour at room temperature, then added to substrate, acting for two days at 38°. The extent of plastein formation is expressed as plastein nitrogen in percentage of the total nitrogen, the nitrogen content of enzyme, activator, and inhibitor having been deducted.

Discussion

It has been shown that papain, acting upon concentrated peptic or papain digests of egg albumin, gives rise to a plastein, a protein-like substance, insoluble in trichloracetic acid, and similar in properties to the peptic plastein described by Wasteneys and Borsook (22). Whatever the nature of the plastein, a problem to be discussed in a following contribution, these experiments clearly demonstrate that active enzyme is necessary for its formation. The activation of the enzyme by hydrogen sulphide, cyanide, and cysteine, and its complete inactivation by copper salts, iodoacetate, and alloxan, show that free SH groups are necessary for the plastein-forming activity, as is true of the hydrolytic activity. The reactivation by hydrogen sulphide of papain inactivated with hydrogen peroxide supports this thesis.

The optimum pH for plastein formation is at pH 4.8, and the plastein may be rehydrolysed by papain, the optimum, not sharply defined, being in the range of pH 4–5. The papain action therefore differs from peptic formation of plastein, where the optimum pH, shown by Wasteneys and Borsook (22) to be at pH 4.0, is higher than the optimum for hydrolysis. It is interesting that the pH range of plastein-forming activity of papain corresponds very closely with that of the synthetic activity of the enzyme, as determined by Bergmann and Fraenkel-Conrat (10). The relation is also similar to the effect of pH upon cathepsin activity, as reported by Eder, Bradley, and Belfer (14).

If plastein formation is a true enzymatic synthesis, it would appear that concentration is the only factor controlling the direction of the reaction: the optimal pH and oxidation-reduction conditions are approximately the same for both changes.

The effect of substrate concentration is very similar to that observed by Borsook and Wasteneys (13) in the case of pepsin, although they obtained a lower minimum concentration—9 to 13 mg. of nitrogen per ml.

The effect of enzyme concentration on rate of plastein formation follows the square-root law, similar to that observed by a number of workers in the

case of peptic hydrolysis. Northrop (18) has suggested that this is due to a combination between enzyme and substrate, and this probably takes place in plastein formation, for Wasteneys and Crocker (24) have indicated that in the peptic formation of plastein the enzyme may be absorbed on the plastein.

It has already been noted that substances that block or oxidize free SH groups inactivate papain. The observation of Voegtlin and co-workers (17, 20) that reducing conditions favour proteolysis, and oxidizing conditions favour plastein formation, has not been confirmed. It may be noted that Strain and Linderstrøm-Lang (19) have recently reported a failure to confirm Voegtlin's results. Under optimal conditions for plastein formation from albumin digests aeration inactivates the enzyme.

The inhibiting effect of hydroxylamine suggests that aldehyde groups may play some role in the activity of papain, as proposed by Bergmann and Ross (6). Waldschmidt-Leitz and Rauchalles (21) have indicated the possibility of an aldehyde group in erepsin. If the square-root relation be applied to the results with hydroxylamine, it is apparent that the enzyme activity is reduced by about one-half.

Phenylhydrazine has no effect on the formation of plastein by Grassmann papain. Bergmann and Fraenkel-Conrat (10) showed that this substance did not depress the synthetic activity of papain in the presence of cysteine. If the enzyme be treated with phenylhydrazine and the latter subsequently removed by benzaldehyde, the plastein-forming activity is reduced by exactly one-half, if the square root law be applied. Bergmann, Fruton, and Fraenkel-Conrat (9) found that papain thus treated completely lost its hydrolytic activity.

The strongly inhibitive action of benzaldehyde is in contrast to the striking stimulation of plastein formation observed by Wasteneys and Borsook (23) in the case of pepsin. No explanation for this difference can at present be offered. A considerable amount of precipitate is formed when benzaldehyde is added to the papain digests even in the absence of active enzyme. It seems probable that this is a condensation product; although Wasteneys and Borsook call it a plastein their data show that its properties are different from the plastein formed by active enzyme.

In comparing the plastein-forming properties of papain and pepsin, the following differences may be noted. Production of plastein by papain takes place at approximately the same pH optimum as does the hydrolysis of plastein by the same enzyme: pepsin requires a higher pH for plastein formation than for hydrolysis. Papain requires free SH groups: pepsin does not. Benzaldehyde enhances the yield of plastein in the case of pepsin, but depresses the yield in the case of papain.

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